From: Hill, William <hillwi@musc.edu>
To: Paul Weinberger <paul.weinberger@ochsnerlsuhs.org>
Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Date: 4/12/2020 9:01:32 PM
Subject: BSL3

EXTERNAL EMAIL: EVALUATE
Guys Eric Meissner just emailed me:

“I have a friend at UNC that works on coronaviruses and has BSL3 capacity, but they are booked10 times over at the moment. Maybe if your LSU BSL2 work goes well than I’d be happy to make the connect with UNC and see where things are at? Timothy Sheahan is my friend there, he works with Ralph Baric’s group“

So if we start to see anything positive we may have an in at the next level.

Dave

William D. Hill, Ph.D.
Sent from my iPhone

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Ikegame, Satoshi <satoshi.ikegame@mssm.edu>
Date: 10/16/2020 7:57:07 PM
Subject: Spike del-Tyr144

So, Kristian Andersen reminded me to check on CoV-Glue for del-Tyr144—he agrees it is interesting! I checked and it has occurred so far other135 times in GISAID. Kinda cool! Will be fun to see the effect on the virus
You can see it here: http://cov-glue.cvr.gla.ac.uk/#/project/deletion/S:nca:21991:21993

I went ahead and ordered the gBlock to put del-Tyr144 into your pEMC-VSV-eGFP-Spike G614 del21 plasmid. Also.. Stan and Ana (his wife, also a faculty here) reminded me that the Incucyte will be able to show us if the virus behaves differently in terms of plaque sizes or growth rates because it can “bin” objects over time, classifying by size etc..

Thanks so much, Satoshi and Benhur.. and sorry for all the emails!

Have a nice weekend, everyone.

Best,

Jeremy

From: Ikegame, Satoshi <satoshi.ikegame@mssm.edu>
Date: Friday, October 16, 2020 at 2:17 PM
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Cc: Mohammed Siddiquey <siddiquey27@gmail.com>, Haas, Griffin (Student) <griffin.haas@icahn.mssm.edu>, Ivanov, Stanimir S. <sivano@lsuhsc.edu>, Lee, Benhur <benhur.lee@mssm.edu>, Kowdle, Shreyas <shreyas.kowdle@mssm.edu>
Subject: Re: Latest VNA data

Dear Jeremy.

I am also excited that our project is going forward.

1; About growth analysis;

Actually I have no experience in VSV (just started 6 month ago).

But I recommend to start from MOI=0.01 (this is general inoculation in fast growing negative strand RNA viruses).

0.001 is too little and result will be much variable depending on the number of initial entry.

(i) Infect virus for one hour, then wash with PBS to remove residual viruses, fill with new medium.

(ii) Collect sup (collect half of sup and fill same amount of new medium) every 12 (or 24) hours until it reaches confluent (3 days?).

(I recommend to follow one time point after infection becomes confluent. Peak titer tends to delay compared to GFP confluency in paramyxoviruses.)

5; Yes. I believe I didn't send D614-del21-VSV plasmid to avoid mistake and I didn't make full length G614. It must be G614-del21.

Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Friday, October 16, 2020 2:10:44 PM
To: Ikegame, Satoshi

Cc: Mohammed Siddiquey; Haas, Griffin (Student); Ivanov, Stanimir S.; Lee, Benhur; Kowdle, Shreyas
Subject: Re: Latest VNA data

Hi Satoshi,

1. This all sounds fantastic. YES of course we are super excited to test the J6, J7, and J21 mutants (T478I, F490L, and N501Y) in our adaptation of your guys' beautiful neutralization assay (using the incucyte).. We can at first test them in separate (non-multiplex) fashion against wild-type in same backbone (eGFP).
    a. But very soon, we will be testing these mutants in 2-plex assay against the wild-type Spike in mScarlet-I or Crimson E2 backgrounds.
    b. We will also test if same MOI of mScarlet and eGFP behave the same in neutralization assay when tested using both as wild-type Spike in 2-plexes.
    c. We can also do growth curves to compare the different color rVSV FP Spike versions..please let us know if you have a favorite protocol (time points, MOI) for this. We don't do VSV growth curves often.

2. Good news: Sounds like the La Dept of Health wants us to get an EUA for this. So our collaboration should grow and provide useful data for public health. I was told that the Dept of Health approved funding to buy us an Incucyte S5 for this work.

3. Also… spoke to my HCMV collaborator / friend Nat Moorman who is a faculty in same Dept with Ralph Baric and Mark Heise.. he works closely with them on antiviral drug screens.. he is gonna ask them if they might be interested in helping us look at any of these new SARS-CoV-2 mutants that we recently sequenced from patients in nursing home etc type outbreaks. (a bunch are in ORF7a ..one in ORF8).. Anyway, he'll tell me later today if they are interested in helping us look at the Spike del-Tyr144 in their mouse adapted SARS-CoV-2 clone.

4. For putting del-Tyr144 into pEMC-eGFP-Spike D614G del21, here's my strategy … igest the pEMC-eGFP-Spike D614G del21 plasmid with MluI plus Bsu36I and use Gibson Assembly to insert the Tyr144 deleted version…see attached gb file for the gBlock I will order

5. JUST TO CONFIRM. The plasmid you sent to Siddiquey and I for inserting the YFP2, BFP2, mScarlet, Crimson E2, etc gBlocks is the D614G del21 version, right? If so that's what I'll put it into.

6. And gosh, co-infection with Influenza A.. what a nightmare!

Cheers,

Jeremy

From: Ikegame, Satoshi <satoshi.ikegame@mssm.edu>
Date: Friday, October 16, 2020 at 9:04 AM
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Cc: Mohammed Siddiquey <siddiquey27@gmail.com>, Haas, Griffin (Student) <griffin.haas@icahn.mssm.edu>, Ivanov, Stanimir S. <sivano@lsuhsc.edu>, Lee, Benhur <benhur.lee@mssm.edu>, Kowdle, Shreyas <shreyas.kowdle@mssm.edu>
Subject: Re: Latest VNA data

I haven't thought of sialic acid binding in coronavirus.

http://biorxiv.org/cgi/content/short/2020.10.14.335893

I found one paper about synergic effect by influenza co-infection (not other viruses).

Neuraminidase may be doing something.

Next week (hopefully on Monday), we will ship next material.

1; T478I mutant in GFP backbone, labeled as J6mut

2; F490L mutant in GFP backbone, labeled as J7mut

3; N501Y mutant in GFP backbone, labeled as J21mut

(To avoid confusion, I don't ship other colored mutant virus now.)

Could you test how neutralization pattern is affected by these viruses?

Then, we can chose final 2 candidates (+ wild type) for our first publication.

I will work on E2-crimson and mScarlet of these mutants.

This will be done in 2-3 weeks.

Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Thursday, October 15, 2020 5:07:34 PM
To: Ikegame, Satoshi
Cc: Mohammed Siddiquey; Haas, Griffin (Student); Ivanov, Stanimir S.; Lee, Benhur; Kowdle, Shreyas
Subject: Re: Latest VNA data

Hi Satoshi,

Our microscopes can resolve all the colors (at least Stan has one that can) and the team from Celigo is gonna Demo us their unit on Nov 2- Nov 5.

Regarding the deletion of Tyr144.. Benhur noted that this is in the Galectin-3 like portion of the S1 NTD.. and might well be interesting because it is involved in binding sugars on the cell surface during attachment (N-acetylneuraminic acid). Benhur seems to think should look at it.. but **of course I know you're doing a lot (A TON) of work right now**.. If it helps set the ground work, maybe I'll just try to develop a gBlock or other type of simple cloning strategy to get it this deletion of Tyr144 into pCAGGS-Spike (Florian's plasmid) or into pEMC VSV Spike d21 CT plasmid..

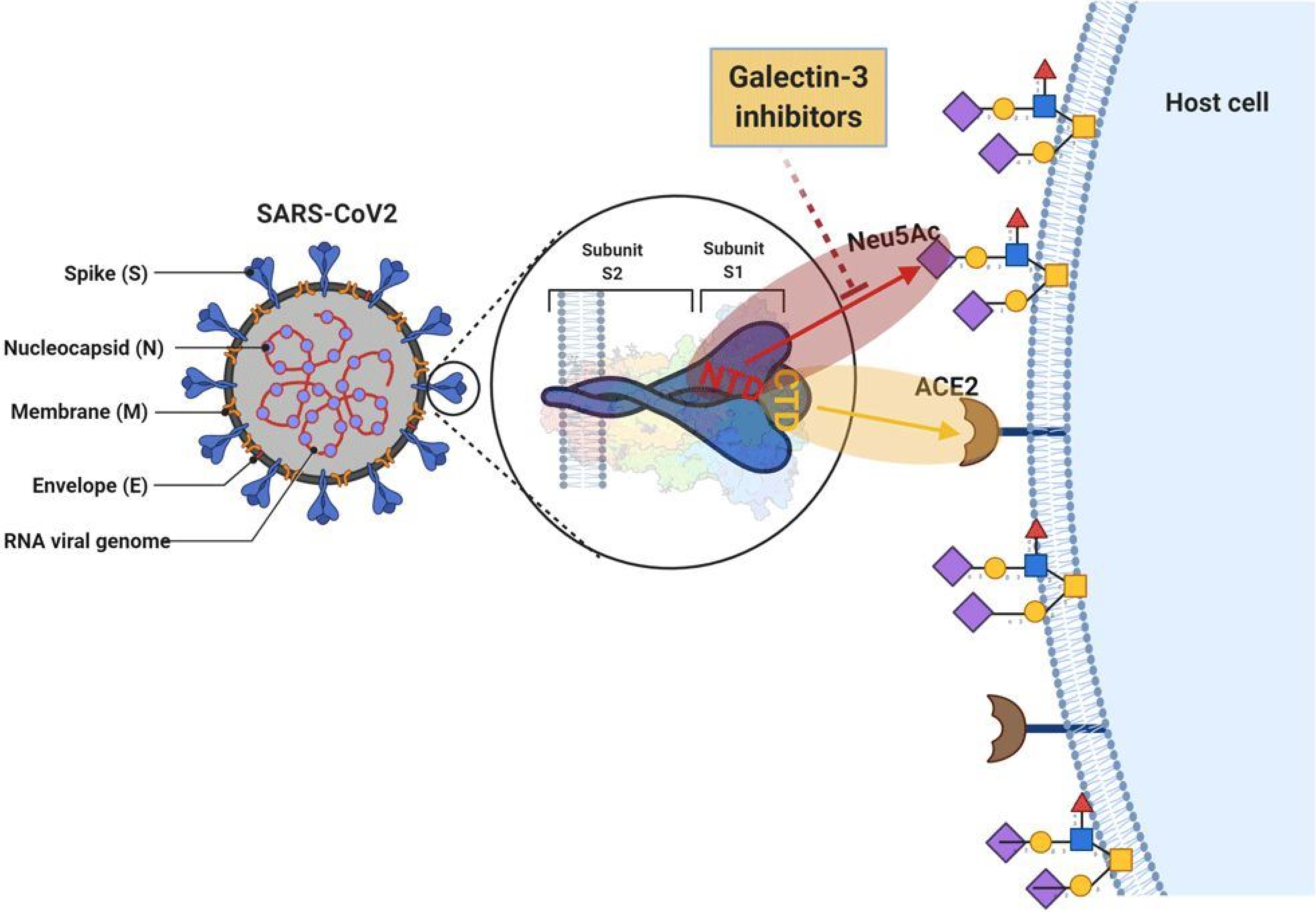
Interesting to note that the two people who were sick from this deletion of Tyr144 virus were medical residents (ages < 35 years old) who work in the same department in our hospital / campus. The CDC N1 and N2 Ct values from their COVID-19 RT-PCR test were ~15! That's pretty low Ct and suggest the mutant is still nasty / pathogenic enough to make people ill.

Here's a cool paper about the Galectin fold https://f1000research.com/articles/9-1078

Excited to grow up your new viruses.

Cheers,

Jeremy


SARS-CoV2
Spike (S)
Nucleocapsid (N)
Membrane (M)
Envelope (E)
RNA viral genome
Subunit S2
Subunit S1
Galectin-3 inhibitors
Neu5Ac
NTD
CTD
ACE2
Host cell

From: Ikegame, Satoshi <satoshi.ikegame@mssm.edu>
Date: Thursday, October 15, 2020 at 9:42 AM
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>, Kowdle, Shreyas <shreyas.kowdle@mssm.edu>
Cc: Mohammed Siddiquey <siddiquey27@gmail.com>, Haas, Griffin (Student) <griffin.haas@icahn.mssm.edu>, Ivanov, Stanimir S. <sivano@lsuhsc.edu>
Subject: Re: Latest VNA data

Dear Jeremy.

I hope your machine can distinguish all colors well, and hope to see merged images with all colored virus infection (like rainbow).

Actually, we are now making different insertion mutants around 200 position in collaboration with other lab. Minor mutants from clinical isolates seemed to become dominant in the mouse.

We are yet to analyze this mutant, but I feel that N-terminus may be relatively flexible and can accept these kind of (presumably neutral) mutations.

https://www.tandfonline.com/doi/full/10.1080/22221751.2020.1806735

One study shows that 2% of genome have deletion. So it is possible that incidental neutral mutants spread locally.

Rather, virus may get benefit by driving human asymptomatic.

However, we can make mutants if this strain continues spreading in your region.

I want to stay and see the situation until my current production finishes (-2weeks) and make final decision.

Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Wednesday, October 14, 2020 6:18:06 PM
To: Kowdle, Shreyas; Ikegame, Satoshi
Cc: Mohammed Siddiquey; Haas, Griffin (Student); Ivanov, Stanimir S.
Subject: Re: Latest VNA data

Hi Satoshi and Shreyas (cc Griffin, Stan, & Siddiquey)

We received the clone 8 VERO TMPRSS2 cells and all the rVSV viruses today.. plenty of dry ice was left.

Thank you so much!!

We are excited to start some multiplexing experiments!!

*By the way we sequenced a bunch of viruses recently and found two from an infection cluster where Tyr144 was deleted (in frame) due to a TTATTA repeat compressing to one copy of TTA (in frame2).. so this eliminates a Tyr (either 144 or 145) since there are usually two Tyr in a row there.

Pretty cool tho because that virus must be viable and able to transmit!   Maybe an easy paper to do?   **What do you guys think?**

These two viruses can be found in GISAID as:

hCoV-19/USA/LA-EVTL607/2020

hCoV-19/USA/LA-EVTL614/2020

Best,

Jeremy

Sent from phone (please forgive typos)

> On Oct 13, 2020, at 3:34 PM, Kowdle, Shreyas <shreyas.kowdle@mssm.edu> wrote:
>
> Apologies Jeremy, but I had already dropped the package in our mail room for pickup.

But I will make a note of this and will use the same for future shipments.

Shreyas Kowdle

Research Technician / Lab Manager, Lee Lab

Department of Microbiology
Icahn School of Medicine at Mount Sinai

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Tuesday, October 13, 2020 3:36:05 PM
To: Kowdle, Shreyas; Ikegame, Satoshi
Cc: Haas, Griffin (Student); Mohammed Siddiquey
Subject: Re: Latest VNA data

Hi Shreyas,

If it is not too late can you change the shipment label so that it reads “BRI F5-33” ? That’s our room #.. F2-33 will get it to our COVID-19 diagnostic lab. It will get to us either way though.

THANKS SO MUCH FOR THIS SHIPMENT!

Cheers,

Jeremy

From: "Kowdle, Shreyas" <shreyas.kowdle@mssm.edu>
Date: Tuesday, October 13, 2020 at 2:15 PM
To: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>, "Ikegame, Satoshi" <satoshi.ikegame@mssm.edu>
Cc: "Ivanov, Stanimir S." <sivano@lsuhsc.edu>, "Lee, Benhur" <benhur.lee@mssm.edu>, "Haas, Griffin (Student)" <griffin.haas@icahn.mssm.edu>, Mohammed Siddiquey <siddiquey27@gmail.com>
Subject: Re: Latest VNA data

Hello Jeremy,

Just finished shipping your package with the following in them:

Virus tubes include:

1. Nanoluc-P2A-GFPin G614 backbone

2. BFPin G614 backbone

3. YFPin G614 backbone

4. mScarletin G614 backbone

5. C2-crimson in G614 backbone

6. J15g

Satoshi is not sure about the titer, but he expects it to be ~ 10^4 IU/ml)

Cells included:

7. VeroCCL81-TMPRSS2 - clone 8

There should be 2 tubes each, totaling 14 tubes in the shipment. The FedEx label is attached and the tracking number is:
8160-0934-4532

Please do let us know once you receive the shipment.

Warm Regards,

Shreyas Kowdle

Research Technician / Lab Manager, Lee Lab

Department of Microbiology
Icahn School of Medicine at Mount Sinai

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Tuesday, October 13, 2020 2:48:30 PM
To: Ikegame, Satoshi
Cc: Ivanov, Stanimir S.; Lee, Benhur; Haas, Griffin (Student); Kowdle, Shreyas; Mohammed Siddiquey
Subject: Re: Latest VNA data

Hi Satoshi,

Just met with Stan to discuss planning next steps.   As soon as you get us the new viruses you mentioned below (like Scarlet F490L and GFP F490L, and or T478I versions, etc), we can move forward to try to get some experiments done for a quick pre-print / paper.. It would be Ikegame et al, maybe with Siddiquey as a second co-first author, but your name first since you developed and rescued all these viruses.

One news is that we now have the 3-color Incucyte device being assembled in our INLeT Core facilty. It should be ready in another two weeks.. so if you'd like to put any other mutants like F490L, T478I, or some other top ranking ones like N501Y into the E2-Crimson that might make sense.   If you have the wild-type Spike in BFP, too, please send it because we can use that for Proof-of-Principle figure in the Preprint / manuscript for this "Rainbow" multiplex Spike variant neutralization system.

Super exciting!

Best,

Jeremy

From: "Ikegame, Satoshi" <satoshi.ikegame@mssm.edu>
Date: Friday, October 9, 2020 at 2:13 PM
To: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
Cc: "Ivanov, Stanimir S." <sivano@lsuhsc.edu>, "Lee, Benhur" <benhur.lee@mssm.edu>, "Haas, Griffin (Student)" <griffin.haas@icahn.mssm.edu>, "Kowdle, Shreyas" <shreyas.kowdle@mssm.edu>, Mohammed Siddiquey <siddiquey27@gmail.com>
Subject: Re: Latest VNA data

Dear Jeremy.

I will ship items hopefully on next Tuesday;

1; Vero TMPRSS2 cells clone 8 (clone purified)

We will check TMPRSS2 expression by qPCR later, but it shows good entry and small syncytia.

I believe this must be real clone.

I think clone purified cells may not be suitable for virus amplification.

Some cells are not infected and syncytia formation is quite weak.

However, entry seems good and small syncytia block the dilution of FP (easy to detect).

Bulk Vero-TMPRSS2 (previously we sent) or Vero cells (with 0.2 ug/ml TPCK-trypsin treatment) may be favorable.

2; CoV2-VSV;

nanoluc-P2A-GFP, BFP, YFP, mScarlet, C2-crimson in G614 backbone.

In addition, virus labeled as 'J15g' (G614 mutant with synonymous mutation (third codon is 'g') at 476 position)

J15g will be control virus to our assay, but nanoluc-GFP will be second control virus in case that we need nanoluc signal.

Recent progress;

- mScarlet-CoV2-VSV (F490L+G614) was rescued and being amplified.
- mScarlet-CoV2-VSV (T478I+G614) was rescued and being amplified.
- GFP-CoV2-VSV (F490L+G614) was amplified.
- GFP-CoV2-VSV (T478I+G614) rescue failed once, but I hope it will be rescued soon.
- 3 RBD mutants are being rescued, but rest of them (>10) were rescued and being amplified (give me 2-3 weeks to prepare all).

I will prioritize T478I-GFP for you to start your designed assay.

Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Saturday, October 3, 2020 12:04:15 PM
To: Ikegame, Satoshi

Cc: Ivanov, Stanimir S.
Subject: Re: Latest VNA data

Hi Satoshi's,

Yes. Looks to me like the antibodies cannot block syncytia but this is largely expected since cell to cell contacts are hard for Antibodies to access and it probably happens super rapidly.

Sent from phone (please forgive typos)

> On Oct 1, 2020, at 5:29 PM, Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:

Dear Jeremy.

Thank you for updating us.

We can find better detection method and cut off line through this.

It is interesting that area size reached peak around 10-12 hrs (first round infection).

Then shows slight reduction (beginning of secondary infection?).

Patient serum is not blocking syncytia formation once infected?

It is good to know the character of our virus.

Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Thursday, October 1, 2020 5:27:56 PM
To: Lee, Benhur; Ikegame, Satoshi; Haas, Griffin (Student)
Cc: Ivanov, Stanimir S.; siddiquey27@gmail.com; Woolard, Matthew D.; Kevil, Christopher
Subject: Latest VNA data

USE CAUTION: External Message.

Hi Benhur, Satoshi, and Griffin, (cc: Matt, Siddiquey, CK, Stan)

Just in case you’d like to see it, here’s the latest snapshot of some new, pretty-much unprocessed raw data from your guys’ beautiful rVSV-[eGFP]-Spike VNA going into the HEK293 TMPRSS2/ACE2 clone on black-sided 96well plates (collage-coated, of course.).

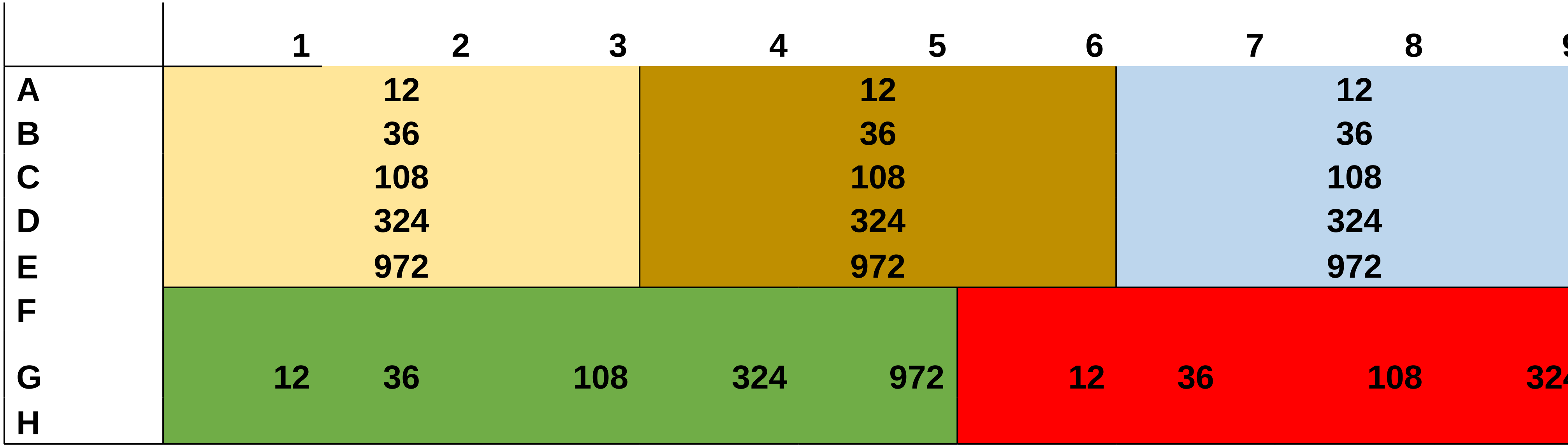
Siddiquey did all the VNA assays, with help in planning the layout from Stan.. Data from Incucyte steps courtesy of Stan and Ana.

Best,

Jeremy

Pasting how the plate is set up below, and including as an excel.

Also.. not sure how useful the “Average GFP object area” is but good to see the data plotted a few different ways.



	1	2	3	4	5	6	7	8	
A		**12**			**12**			**12**	
B		**36**			**36**			**36**	
C		**108**			**108**			**108**	
D		**324**			**324**			**324**	
E		**972**			**972**			**972**	
F									
G	**12**	**36**	**108**	**324**	**972**	**12**	**36**	**108**	**32**
H									

From: "Ivanov, Stanimir S." <sivano@lsuhsc.edu>
Date: Thursday, October 1, 2020 at 4:08 PM
To: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>, "Siddiquey, Mohammed N." <msidd2@lsuhsc.edu>, "Dragoi, Ana Maria" <adrag1@lsuhsc.edu>

Subject: Latest data from the last two plates

Hi Jeremy,

Here is the latest data summary for the two plates we are currently running. We are going to pull them out tomorrow.

Anyway, LS031 has phenomenal neutralization capacity (see plate A - rows FGH, 6 through 10) - almost a complete block even @ 1:972 dilution. Also, plate B colomn12 had less cells seeded, which accounts for the data variation. Other than that, the data and the new distribution format looks good...

Next week, we should try setting up a 384 well plate to test how well the assay will work in that format.

Cheers,

Stan

Stanimir Ivanov

Assistant Professor

Department of Microbiology and Immunology

1501 Kings Highway, B 2-221

Shreveport, LA 71103

Ph (318) 675-3209

sivano@lsuhsc.edu

http://www.lsuhscshreveport.edu/microbiology

From: reneau youngblood <reneauy@hotmail.com>
Sent: Thursday, October 1, 2020 14:58
To: Ivanov, Stanimir S. <sivano@lsuhsc.edu>
Subject: Incucyte Data

EXTERNAL EMAIL: EVALUATE

Sent from Outlook

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Dan Snyder <dan@migscenter.com>
Date: 10/16/2020 5:41:34 PM
Subject: Re: Plates of RNA samples..
Attachments: Coded Facility name_mutations_20201015 ORF7a Spike etc.xlsx
EPI_ISL_402124 WIV4.gb

Hi Dan, (also copying Vaughn and my superb collaborator Benhur Lee so they can have this info, too)

This is great news! Now I'll check if I can find a BSL-3 CoV2 lab that might be willing to transfect them. Attaching a file with the plate and well info + a .gb file that annotates the reference sequence with the mutations.

ONE IDEA.. if we do find a BSL3 collaborator to transfect them, we can even pool together each set of samples that has an identical virus in it, which might help increase success of recovery..

My friend Nat Moorman (Assoc Prof at UNC CH) is in same Dept and collaborates with Ralph Baric and Mark Heise.. They might help us look at some of these in a mouse model… Though he suggested that the UNC folks can just put the mutations into a mouse-adapted infectious clone.

EVTL607 and 614 are identical to each other, so that's like 10-16 uL..
EVTL 410-417 (8 viruses) are identical to each other… so that gives at least 40 uL
EVTL 437-438 are identical too, so that's like 10-16 uL..

Crossing fingers that we can find someone to rescue these.. (assuming that I'm right that this is worth doing .. haha).. seems like a good insurance policy to maximize impact of any epi-papers re these viruses the outbreaks we found them from…

Best,

Jeremy

From: Dan Snyder <dan@migscenter.com>
Date: Friday, October 16, 2020 at 12:01 PM
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Subject: Re: Plates of RNA samples..

EXTERNAL EMAIL: EVALUATE
Hi Jeremy,

We do still have the original RNA plates, which were immediately frozen again after we pulled our aliquot. I would guess we have around 5-8uL or RNA left in each well.

Dan

On Fri, Oct 16, 2020 at 1:36 AM Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:

> Hi Dan,
>
> There are about 12 samples from the most recent set of 4 plates you guys just sequenced from that are potentially worth growing up. Found out that our testing lab tossed out all the remaining original viral transport media samples corresponding to those from the -80 freezers.

> Just wondering if you guys still have any of the RNA left … in case I can find a BSL-3 CoV2 collaborator who wants to try to transfect it into VERO cells.
>
> Long shot.. but just curious. (I'm furious that our testing lab threw the original swab + VTM samples away.. Argh!)
>
> Cheers,
>
> Jeremy
>
> Sent from phone (please forgive typos)

--
Dan Snyder, Director
Microbial Genome Sequencing Center
355 Fifth Avenue, Suite 810
Pittsburgh, PA 15222
(412) 475-2318

From: Calvert, Tracy <THaile@lsuhsc.edu>
To: Haacker, William <BHaack@lsuhsc.edu>
White, Valarie M. <vwhit5@lsuhsc.edu>
Date: 10/12/2020 5:16:02 PM
Subject: RE: Final Invoice for Year 2 Univ. of Arizona Subcontract (1R01AI143191-01)

Thanks!

Tracy H. Calvert
Director
Louisiana State University Health Sciences Center
Office of Sponsored Programs & Tech Transfer
1501 Kings Highway
BRI, Suite F1-53
Shreveport, LA 71103
318-675-7884 phone
318-675-7015 fax
thaile@lsuhsc.edu

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From: Haacker, William <BHaack@lsuhsc.edu>
Sent: Monday, October 12, 2020 9:48 AM
To: Calvert, Tracy <THaile@lsuhsc.edu>; White, Valarie M. <vwhit5@lsuhsc.edu>
Cc: Kamil, Jeremy P. <jkamil@lsuhsc.edu>; Nelson, Annella <ANELSO@lsuhsc.edu>
Subject: RE: Final Invoice for Year 2 Univ. of Arizona Subcontract (1R01AI143191-01)

Tracy,

August books closed last week and we’re in the process of invoicing. We’ll move this one to the top and let you know when completed.

Thanks!
Bill

From: Calvert, Tracy <THaile@lsuhsc.edu>
Sent: Monday, October 12, 2020 8:04 AM
To: Haacker, William <BHaack@lsuhsc.edu>; White, Valarie M. <vwhit5@lsuhsc.edu>
Cc: Kamil, Jeremy P. <jkamil@lsuhsc.edu>; Nelson, Annella <ANELSO@lsuhsc.edu>
Subject: FW: Final Invoice for Year 2 Univ. of Arizona Subcontract (1R01AI143191-01)

Bill & Valarie,

Please see initial email below. This is for Dr. Kamil’s subaward with the University of Arizona. Can you let us know if all invoices have been submitted for his grant year 02, PS account 110251222A.

thanks,
Tracy

Tracy H. Calvert
Director
Louisiana State University Health Sciences Center

Office of Sponsored Programs & Tech Transfer
1501 Kings Highway
BRI, Suite F1-53
Shreveport, LA 71103
318-675-7884 phone
318-675-7015 fax
thaile@lsuhsc.edu

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From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Thursday, October 8, 2020 4:53 PM
To: Calvert, Tracy <THaile@lsuhsc.edu>; Shv Grants Administration <Grants@lsuhsc.edu>
Cc: Nelson, Annella <ANELSO@lsuhsc.edu>
Subject: Final Invoice for Year 2 Univ. of Arizona Subcontract (1R01AI143191-01)

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The email below from Sean Cadin (University of Arizona) is regarding the subcontract we have on1R01 AI143191, which I am a PI on with Dr. Nat Moorman (UNC Chapel Hill) and Dr. Felicia Goodrum (she is the contact PI and is based at U of AZ).

Please let me know if I can of assistance in any way, and see below for further details.

Cheers,

Jeremy

From: Sean Cadin <scadin@bio5.org>
Date: Thursday, October 8, 2020 at 4:02 PM
To: "Moorman, Nathaniel John" <nathaniel_moorman@med.unc.edu>, "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
Cc: Felicia Goodrum <fgoodrum@arizona.edu>, "Calvert, Tracy" <THaile@lsuhsc.edu>, "Baric, Toni C" <antoinette_baric@med.unc.edu>
Subject: Re: [EXT]Fw: 5112236 Moorman

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Sean M Cadin
Business Manager, Senior
BIO5 Institute
THE UNIVERSITY OF ARIZONA

***I will be working remotely until further not

TW Keating Bioresearch Bldg., 102
PO Box 210240 | Tucson, AZ 85721

Office: 520-626-4680
scadin@email.arizona.edu

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> Thomas W. Keating Bldg, Rm 425
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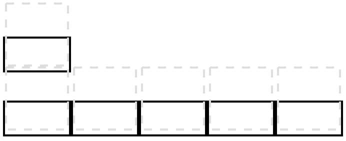

From: Calvert, Tracy <THaile@lsuhsc.edu>
To: Sean Cadin <scadin@bio5.org>
Date: 10/12/2020 4:00:51 PM
Subject: RE: [EXT]Fw: 5112236 Moorman

Hi Sean, I have inquired with our Grants Accounting office – thanks!
Tracy

Tracy H. Calvert
Director
Louisiana State University Health Sciences Center
Office of Sponsored Programs & Tech Transfer
1501 Kings Highway
BRI, Suite F1-53
Shreveport, LA 71103
318-675-7884 phone
318-675-7015 fax
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> 919-699-0415 (personal cell)

From: Haacker, William <BHaack@lsuhsc.edu>
To: Calvert, Tracy <THaile@lsuhsc.edu>
White, Valarie M. <vwhit5@lsuhsc.edu>
Date: 10/12/2020 2:47:45 PM
Subject: RE: Final Invoice for Year 2 Univ. of Arizona Subcontract (1R01AI143191-01)

Tracy,

August books closed last week and we're in the process of invoicing. We'll move this one to the top and let you know when completed.

Thanks!
Bill

From: Calvert, Tracy <THaile@lsuhsc.edu>
Sent: Monday, October 12, 2020 8:04 AM
To: Haacker, William <BHaack@lsuhsc.edu>; White, Valarie M. <vwhit5@lsuhsc.edu>
Cc: Kamil, Jeremy P. <jkamil@lsuhsc.edu>; Nelson, Annella <ANELSO@lsuhsc.edu>
Subject: FW: Final Invoice for Year 2 Univ. of Arizona Subcontract (1R01AI143191-01)

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To: Haacker, William <BHaack@lsuhsc.edu>
White, Valarie M. <vwhit5@lsuhsc.edu>
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>
> Get Outlook for Android
>
> ---
>
> **From:** Best, Lisa M <lisa_best@med.unc.edu>
> **Sent:** Thursday, October 8, 2020 10:19:44AM
> **To:** Baric, Toni C <antoinette_baric@med.unc.edu>
> **Subject:** 5112236 Moorman
>
> Hey Toni…………wanted to check in to see where we stood with Nat’s University of Arizona subcontract that ended 8/30/20. Salaries have gone into suspense so I have to make a move.
> Thanks
> L
>
> ____
> Lisa M Best
> University of North Carolina
> Microbiology and Immunology
> Accounting
> 919-699-0415 (personal cell)

From: Sean Cadin <scadin@bio5.org>
To: Moorman, Nathaniel John <nathaniel_moorman@med.unc.edu>
Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Date: 10/8/2020 9:02:12 PM
Subject: Re: [EXT]Fw: 5112236 Moorman

EXTERNAL EMAIL: EVALUATE
Hello all -

Apologies for the delay. Thought I would put this out for everyone - we are working on the next year of the award (extending your subawards).. We are a little short staffed and trying to play catch up. Please know that these amendments are one of my top priorities and will let you know when they are enroute. In the meantime, have you already submitted your final invoices for year 2 of the award? Please let me know.

I will hopefully let you know about these soon - please don't hesitate to contact me with questions.

Sean M Cadin
Business Manager, Senior
BIO5 Institute
THE UNIVERSITY OF ARIZONA

*****I will be working remotely until further**

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scadin@email.arizona.edu

www.arizona.edu
facebook | twitter | instagram | linkedin

On Thu, Oct 8, 2020 at 1:53 PM Goodrum Sterling, Felicia D - (fgoodrum) <fgoodrum@arizona.edu> wrote:

> Hey Nat, I really cannot but maybe Sean can. I have copied him. Thanks, Felicia
>
> Felicia D. Goodrum, PhD
> Professor, Immunobiology
> BIO5 Institute
> University of Arizona
> 1657 E. Helen St.
> Thomas W. Keating Bldg, Rm 425
> Tucson, AZ 85721
>
> (520) 626-7468
> fgoodrum@email.arizona.edu
>
> > On Oct 8, 2020, at 1:49 PM, Moorman, Nathaniel John <nathaniel_moorman@med.unc.edu> wrote:
> >
> > **External Email**
> >
> > Hope all is well! Can you help me resolve the subcontract issue for our R01? (see below). Thanks!

From: Baric, Toni C <antoinette_baric@med.unc.edu>
Sent: Thursday, October 8, 2020 10:36 AM
To: Moorman, Nathaniel John <nathaniel_moorman@med.unc.edu>
Subject: Fwd: 5112236 Moorman

Hi Nat
Can you reach out to Felicia regarding the amendment to your subcontract for year 3. Also in the interim, can you dl the NOA and send to Lisa Best so she can get a LOG?
Thanks
Toni

Get Outlook for Android

From: Best, Lisa M <lisa_best@med.unc.edu>
Sent: Thursday, October 8, 2020 10:19:44 AM
To: Baric, Toni C <antoinette_baric@med.unc.edu>
Subject: 5112236 Moorman

Hey Toni............wanted to check in to see where we stood with Nat's University of Arizona subcontract that ended 8/30/20. Salaries have gone into suspense so I have to make a move.
Thanks
L

_____
Lisa M Best
University of North Carolina
Microbiology and Immunology
Accounting
919-699-0415 (personal cell)

From: mbio@asmusa.org
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Date: 10/5/2020 7:30:46 PM
Subject: Final Decision made for mBio02319-20

EXTERNAL EMAIL: EVALUATE
Dear Dr. Kamil:

Here is a copy of the decision letter for manuscript mBio02319-20 ("In plain sight: the role of alpha-1-antitrypsin in COVID-19 pathogenesis and therapeutics.") by Kasopefoluwa Oguntuyo, Christian Stevens, Mohammed Siddiquey, Robert Schilke, Matthew Woolard, Hongbo Zhang, Joshua Acklin, Satoshi Ikegame, Chuan-tien Hung, Jean Lim, Robert Cross, Thomas Geisbert, Stanimir Ivanov, Jeremy Kamil, and Benhur Lee, for which you were a Contributing Author.

Sincerely,

Arturo Casadevall
Editor in Chief, mBio

Subject: mBio02319-20 Decision Letter

October 5, 2020

Prof. Benhur Lee
Icahn School of Medicine at Mount Sinai
Microbiology
One Gustave L. Levy Place, #1124
New York, NY 10029

Re: mBio02319-20 (In plain sight: the role of alpha-1-antitrypsin in COVID-19 pathogenesis and therapeutics.)

Dear Prof. Lee:

We have completed our review of your manuscript, and I regret to inform you that we will not be able to accept it for publication in mBio. While our review indicated that the manuscript is not suitable in its current state, the work itself seems to be sound. With the necessary revisions, this manuscript could be reconsidered.

Our policy is to offer streamlined decisions and to never request extensive modifications or additional experiments; so for now, our decision must be a rejection. If in the meantime you choose to make the revisions on your own, we are willing to consider the work for one more round of review.

Thank you for your interest in mBio.

Sincerely,

Arturo Casadevall
Editor in Chief, mBio
mBioEditorInChief@asmusa.org
(Signing for the editors)

Editor comments:

The authors have made a novel and potentially exciting observation about the potential role of alpha antitrypsin in inhibiting SARS CoV-2 entry. However, both reviewers identify a number of weaknesses - demonstrating which protease is inhibited, use of both authentic and pseudotyped viruses to demonstrate inhibition, the level of AAT in the lungs and other key tissue surfaces that are relevant for infection in vivo and so on. Although there are numerous weaknesses, it is my sincere hope that the authors will be able to address them in a revised manuscript.

Reviewer #1:

Oguntuyo and colleagues investigated the role of alpha 1 antitrypsin (AAT1) in SARS-CoV-2 entry into target cells. The study was motivated by the finding that sera from SARS-CoV-2 naï¿½ve human donors reduced entry driven by the spike protein of SARS-CoV-2, raising the possibility that these sera contain a cellular factor that blocks entry. The authors suggest that AAT1 is a candidate. While inhibition of SARS-CoV-2 entry by AAT1 is of interest, several points remain open.

Major

It is essential to characterize the contribution of AAT1 to inhibition of SARS-CoV-2 entry by sera from SARS-CoV-2 naï¿½ve individuals. Depletion experiments with AAT1 antibodies are one option to address this point.

Three proteases can contribute to SARS-CoV-2 entry. Cathepsin L can mediate entry into some cell lines but is unlikely to play a role in SARS-CoV-2 spread in the human lung. Moreover, TMPRSS2 can activate the viral spike protein for entry into human lung cells and this depends on pre-cleavage of spike by furin. It is essential to determine which of these proteases are inhibited by AAT1 and this should be done by studying pseudotype entry and by using recombinant proteins.

Even upon trypsin pre-treatment SARS-CoV-2 spike protein-driven entry into Vero cells should be cathepsin L-dependent and also entry into 293T cells expressing ACE2 should depend on cathepsin L. Why was one inhibited by AAT1 but not the other? Along these lines, entry into 293T-ACE2 + TMPRSS2 cells can be mediated by cathepsin L or TMPRSS2, which accounts for the relatively moderate entry inhibition by Nafamostat. This should be clearly stated and data should be confirmed in a cell system in which entry solely depends on TMPRSS2, ideally human lung cells.

It should be demonstrated that AAT1 inhibits entry of authentic SARS-CoV-2.

Minor

It is suggested to revise the title: "In plain sight: the role 1 of alpha-1-antitrypsin in COVID-19 pathogenesis and therapeutics". The authors neither studied SARS-CoV-2 pathogenesis nor did they explore whether AAT1 can be used for COVID-19 therapy.

Exogenous and endogenous proteases. The word exogenous is frequently used to describe proteins expressed upon transfection. It is proposed to replace "exogenous" by "secreted".

"protease-mediated entry" suggests that there this also non-protease mediated entry, which is not the case. Rewording is suggested.

"coronavirus disease 2019 (COVID-19) pathogenicity, SARS-CoV-2 tissue restriction" "Pathogenicity" should be replaced by "pathogenesis", "restriction" should be replaced by "tropism"

"The COVID-19 pandemic that began in December 2019 has resulted in tens of millions of cases with hundreds of thousands of deaths." Precise numbers would be preferable.

The introduction should provide background on AAT1.

"These conditions were designed noting that proteolytic activation of CoV2-S is required for the receptor-induced conformational changes that result in virus-cell membrane fusion and viral entry." This statement is incorrect. It is believed that receptor binding results in conformational changes that promote spike cleavage and cleavage is likely sufficient to trigger membrane fusion.

FIG. 1B must be replaced by real data.

Reviewer #2:

This report is based on the isolated observation is that when trypsin is used as the source of the protease required to activate SARS-CoV2-GP-dependent infection, the presence of a trypsin inhibitor, alpha-1 antitrysin, inhibits infection. The effect of AAT-1 on bona fide virus infection is much less significant. The biological relevance of this finding is not established. AAT-1 is highly abundant in the lung and the authors show that AAT-1 inhibits TMPRSS2-dependent infection but the biological significance of this is not backed up by measurements of AAT-1 in lung. not sure if the authors argue that plasma AAT-1 may function to restrict the tropism of the virus to the lung. The authors suggest that AAT-1 polymorphisms (that are associated with lung disease) may determine susceptibility to SARS-CoV-2 but they do not test this point. In this regard, Fig 4 and associate comments about AAT-1 dependent effects on virus inflammation and coagulation are gratuitous. A potentially interesting findings is that observation that TMPRSSA-2-dependent infection is AAT-1 sensitive but the data is presented as percent of control and so precise effect of AAT-1 cannot be assessed. There is no way to ascertain if the concentration of AAT-1 in the oropharynx or alveolar fluid is comparable to the the concentration that inhibits TMPRSS-2-dependent entry. this is important because the effect using real virus is modest. In summary, this report is based on a single observation under in vitro conditions using pseudotyped virus particles and does not make a convincing link to virus tropism for the lung or for a role of AAT-1 polymorphism in virus pathogenesis.

Staff comments:

If you decide to resubmit this article to mBio after revisions, please use the "Submit Resubmission" link on your user home page. Also, whether resubmitting to mBio or another ASM journal, please reference the previous manuscript control number in your cover letter. These steps will help us to appropriately direct your new manuscript.

***Note that you will need to address the following issues before submitting your revised files:
-Each item in the supplement should be submitted as a separate file (e.g., multiple items should not be combined in a single PDF).

-It appears that more than one author is listed as the first author, or has provided equal contribution, in the footnote on the title page. If more than one co-first author is designated, authors are required to state how the order of names was decided. Please provide an explanation of how the order of names was determined as a second sentence in the footnote. You can refer to the Authorship Guidelineshttps://journals.asm.org/content/authorship-0 for specific information about the requested corrections.

A resubmission of this work to mBio must contain substantial revisions that address the concerns raised in the review.

Please contact us if you have any questions.

Maisha Miles
Managing Editor, mBio

Rob Arthur
Assistant Managing Editor, mBio

mBio@asmusa.org

American Society for Microbiology
1752 N St. NW
Washington, DC 20036
(202) 737-3600

From: Muggeridge, Martin I <MMugge@lsuhsc.edu>
To: Weinberger, Paul M. <pweinb@lsuhsc.edu>
Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Date: 3/20/2020 10:50:30 PM
Subject: Re: Hydroxychloroquine for SARS2

Not doubting clinical effect, but dubious about mechanism of action.

Did some checking and found a paper showing inhibition of SARS-2 entry by hydroxychloroquine.
https://www.nature.com/articles/s41421-020-0156-0

The potential problem with their results is that they used Vero E6 cells, which previous work on MERS showed probably don't have the same pathway of virus entry as human cells.
https://www.ncbi.nlm.nih.gov/pubmed/30413791

The MERS spike protein has two sites that need to be cut by proteases in order for it to cause membrane fusion.
SARS-2 spike also has these two sites.

For MERS spike, the first site (S1/S2) is cut by furin in the cell in which the protein is synthesized.
The second site (S2') is cut by a protease called TMPRSS2 on the surface of target cells, and then membrane fusion happens.

Vero E6 cells don't have TMPRSS2, so the MERS spike protein isn't activated at the cell surface.
Instead, the virus is endocytosed, and the equivalent cleavage is then done by cathepsin L in endosomes.
Cathepsin L needs low pH for activity, so inhibition of entry into Vero E6 cells by hydroxychloroquine would be expected.

There are papers coming out in Cell which show that SARS-2 spike cleavage is done in the producing cell and then by TMPRSS2 on target lung cells.
It can be blocked by camostat mesylate, an inhibitor of TMPRSS2.
Therefore would not expect entry to be greatly affected by hydroxychloroquine, as endocytosis not needed for entry into these cells.

What else could it be doing I wonder.

Martin.

On Mar 20, 2020, at 11:58 AM, Weinberger, Paul M. wrote:

> Have not read any of the primary evidence, just going off WHO recommendations. They list it as one of the current 3 treatment recommendations. Would assume there is at least some evidence to support. Well worth looking into. Just haven’t myself.
>
> -pw
>
> > On 3/20/20, 11:55 AM, "Muggeridge, Martin" <MMugge@lsuhsc.edu> wrote:
> >
> > Hi Paul
> >
> > Have you seen any evidence that it works? Awesome if true.

> If so, I would be really interested to read it.
>
> Chloroquine (and presumably hydroxychloroquine) inhibits entry of some viruses because it raises the pH in endosomes.
> Most coronaviruses enter cells by fusion with the plasma membrane (some of them can enter by endocytosis as a backup if the primary mechanism doesn't work).
> The sequence of the SARS-CoV-2 spike protein suggests it should be able to enter cells by fusing with the plasma membrane, which would mean that hydroxychloroquine would have to have a different mechanism of action than usual, if it works.
>
> Martin M.
>
> On Mar 19, 2020, at 10:59 PM, Weinberger, Paul M. wrote:
>
> > Agree – we have several docs already self treating.
> > It is almost out of stock worldwide as a result.
> >
> > I wonder if Dr. Reddy's is tooled up (OR COULD BE) to produce it quickly?
> >
> > -pw
> >
> > On 3/19/20, 10:51 PM, "Jeremy Kamil" <jeremy.kamil@gmail.com> wrote:
> >
> > *EXTERNAL EMAIL: EVALUATE*
> > Many of you may already be up to speed on it, but this particular antimalarial drug is looking great in terms of promise * price * already approved and great oral bioavailability. (Not "chloroquine" so much as the hydrox- derivative).
> >
> > Probably interferes with entry. 600 mg once a day maintenance dose for post exposure prophylaxis. . Maybe 200 mg b.i.d would be wise for high risk individuals, like ER and critical care docs/ nurses etc.
> >
> > VINCENT RACANIELLO's Thread about latest results:
> > https://twitter.com/profvrr/status/1240786150803062784?s=21
> >
> > CLINICAL TRIAL AT UNIVERSITY OF MINNESOTA
> > https://clinicaltrials.gov/ct2/show/NCT04308668
> >
> > Stay healthy my friends,
> >
> > Jeremy
> >
> > --
> > Sent from Gmail Mobile

From: McGee, David <DMcGee@lsuhsc.edu>

To: McGee, David <DMcGee@lsuhsc.edu>
Vutukuri Amarnath, Geetika Sruti <gvutuk@lsuhsc.edu>
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Muggeridge, Martin I <MMugge@lsuhsc.edu>
Schaal, Danielle L. <dschaa@lsuhsc.edu>
Brown, Kellie N. <kbro38@lsuhsc.edu>
Cartelle Gestal, Monica <mcar17@lsuhsc.edu>
O'Callaghan, Dennis J <DOcall@lsuhsc.edu>
Bodily, Jason M. <jbodil@lsuhsc.edu>
Tatchell, Kelly <KTatch@lsuhsc.edu>
Mosher, Bailey S. <bsnave@lsuhsc.edu>
Shakya, Akhalesh K. <ashaky@lsuhsc.edu>
Scott, Rona S <RScott1@lsuhsc.edu>
Bienkowska-Haba, Malgorzata B. <MBienk@lsuhsc.edu>
Siddiqa, Abida <asidd2@lsuhsc.edu>
Zwolinska, Katarzyna <kzwoli@lsuhsc.edu>
Domma, Anthony J. <adomma@lsuhsc.edu>
Ivanov, Stanimir S. <sivano@lsuhsc.edu>
Bamgbose, Temitayo T. <tbamgb@lsuhsc.edu>
fc72ec5a-999a-49ba-a62d-882486cddc55@anonymous.invalid
Henderson, Lauren A. <lhen10@lsuhsc.edu>
anonymous@anonymous.invalid
Kamil, Jeremy P. <jkamil@lsuhsc.edu>

Date: 11/20/2020 4:55:34 PM

Subject: Skype Department of Microbiology & Immunology Special Seminar: Dr. Hongyan Guo

---

anonymous@anonymous.invalid [8:48 AM]:

I can hear you.

Muggeridge, Martin [9:45 AM]:

Virus binding (for example, EBV) to Nrp1 has been shown to trigger entry via a pathway resembling macropinocytosis. Have you looked at this?

anonymous@anonymous.invalid [9:51 AM]:

Another question about Nrp1, doesnt it bind to furin-cleaved substrates? Could that be why antibodies and soluible nrp1 reduce infection, competition with cell surface NRP1?

Muggeridge, Martin [9:53 AM]:

Yes Tim, that's right. I wonder what would happen with a virus with the furin cleavage site in gB mutated. Would it still bind Nrp1?

anonymous@anonymous.invalid [9:56 AM]:

Okay, thank you.

From: Cooper, Vaughn S. <vaughn.cooper@pitt.edu>
To: Kevil, Christopher <CKevil@lsuhsc.edu>
Date: 1/28/2021 2:08:36 PM
Subject: Re: Sequencing

EXTERNAL EMAIL: EVALUATE
Sorry, late reply.

Regrettably I'm being kept out of the loop about some of the local sequences, but I've not heard any reports of B117 here yet. That said, we are barely looking.

VC

> On Jan 27, 2021, at 7:32 AM, Kevil, Christopher <CKevil@lsuhsc.edu> wrote:
>
> Right Jeremy. Great points all around.
>
> One question I have for Dan and Vaughn, have you guys seen the UK variant in Pitt or in sequences from other regions? If so, how prevalent are they?
>
> It seems to me that the 'UK variant' itself would change over time and host thus making it difficult to really say 'yes or no' where it has been.
>
> I agree with Jeremy that there are US variants in the mix challenging spread of other variants.
>
> Best,
>
> Chris
>
> ---
>
> From: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
> Date: Wednesday, January 27, 2021 at 5:51 AM
> To: Dan Snyder <dan@migscenter.com>
> Cc: Vaughn Cooper <vaughn.cooper@pitt.edu>, Chris <CKevil@lsuhsc.edu>
> Subject: Sequencing
>
> Hi Dan,
>
> Looping in our Vice chancellor, Chris Kevil, here. I think everyone agrees that the Hyb Enrichment Seq you guys are doing is excellent / gives such nice results. I'm not sure when / if we may like to try out an ARTIC protocol, but assuming y'all might offer it down the road, please let us know about pricing and availability.
>
> 1. It sounds like La Dept of Health might come be able to support some of our sequencing going forward.. maybe only about 200 sequences per month? Dr. Kevil will know more re the details.
>
> 2. In CONFIDENCE — preliminary analyses (Pangolin and Spike aa mutation scanning) suggest there may be a few interesting "variants" in the latest 367 sequences you just finished and uploaded to Box. I'm seeing ones with Spike P681H, Q677H, and Q677P, alongside unique constellations of other clade / lineage defining polymorphisms. The first two of those amino acid changes in Spike are already implicated as being "of concern" since these are close to the furin cleavage site aka "polybasic" PRRAR motif. However, I did not see any classic B1.1.7 / 501.Y2 "UK" variant.. yet. (The UK variant does have P681H.. the COG-UK team call that mutation "Pooh" like as in "Winnie the Pooh," similarly they call D614G, "Doug", and N501Y "Nelly").
>
> My suspicion is that there could just as well be USA homegrown variants could be competing fine against the B.1.1.7. Perhaps explaining why it hasn't begun to take over yet? Shreveport might not be NYC or Los Angeles,

but we are along a major travel corridor (I-20 / I-49) so I think we are well poised to pick it up.   I'm just glad we are NOT explicitly pre-screening for B.1.1.7 by S amplicon drop out RT-qPCR because that IMO is garbage for Gen-Epi (adds a huge bias and removes ability to detect homegrown variants that might be out competing others).

Anyway, I'll paste in the "PS" section below some Dropbox links to the csv outputs of some crude web tool analyses I ran last night on the data, in case anyone wants a quick peek. At some point, I'm gonna have to start writing like a half dozen manuscripts, and of course you and Vaughn are on all of them.

Cheers,

Jeremy

PS:

https://www.dropbox.com/s/2bbzfgokbb8kwzd/muts_summ_20210127.rtf?dl=0

https://www.dropbox.com/s/5sg7dg8swq806q4/results_Pangolin_COG_UK_20210127.csv?dl=0

https://www.dropbox.com/s/l4hiyii3dwom6zr/myresults.csv?dl=0

Sent from phone (please forgive typos)

From: Lee, Benhur <benhur.lee@mssm.edu>
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Date: 1/28/2021 8:15:33 AM
Subject: Curr Opinions in Virology Review
Attachments: Current Opinion in Virology invitation and submission instructions.pdf

---

Hi Jeremy,

This is a big ask, but I got the editor to agree to a change in topic.

Richard Plemper is in charge of a special issue in Current Opinions in Virology dedicated to SARS-CoV-2. I had agreed back in Nov to write a review a short one (2,000 words) on the controversy surrounding convalescent plasma.

But since we have spent so much time on collating and trying to understand these variants, I asked Richard if I could change the topic and write something on these variants. He agreed.

I was wondering if you would write it with me. A natural break-down would be genetics and function. You seem to have to have kept track of the evolution and lineage diversification ever since last Fall when we started making these point mutants. I only recently got into it from the structure-function side.

We would both be corresponding authors. You can go first or last, I don't care. The only triock is that the review is due in a week, but it's only 2,o00 words, I already made up the structural figures . . . you can explain the genetics of these strains and perhaps clarify the nomenclature. This is an *OPINION* piece rather than a straightforward review, so they want us to take a stand.

I figured it would be good place to ask for more national genomics sequencing as well as to opine on how to characterize these mutants properly.

I hope you're interested, and we can Skype briefly tomorrow? (*Curr Op in Virol* is a who's who in Virology, so it'll be a good venue for our work).

Best Regards,

Benhur

Benhur Lee, M.D.
Professor of Microbiology
Ward-Coleman Chair in Microbiology
Icahn School of Medicine at Mount Sinai
One Gustave L Levy Place #1124
New York, NY 10029
Office: (212) 241-2552 | Lab: (212) 241-9960

Lab Webpage: LeeLabVirus.Host **| Twitter** @VirusWhisperer

> On Jan 27, 2021, at 7:08 PM, Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:
>
> Great news re the shipment and re: the better titer!   We are gonna get there soon!
>
> Also.. not to distract anyone but I'll gonna drop something in the PS.. we may have a Louisiana "homegrown" variant here.. If any one of you knows how to model a Q677P into the spike, I'd love to know your thoughts as to potential effects on proteolytic processing of the PRRAR motif at 681-685.

Jeremy

PS:

Keep this to yourselves, but potentially interesting lineage /variant.. showing up in sequences from all sorts of different collection sites (community testing, jail, nursing homes).. all appear to be B1.2 GH / Nextstrain 20G.. we saw this mutation in our early Jan upload (viruses from up to Dec 18) but I didn't look closely how much there is.. now I realize it is a lot.. it *may* be out-competing others. It's about 15% of all our viruses in the latest batch. Currently there are only 324 of these Q677P mutants on GISAID (from all over the world), and 166 many of them were collected / sequenced by EVTL lab.. 167 total from Louisiana (Air Force sequenced one, too). We are about to add another 56 from our latest sequencing (it is still being "accessioned"/ prepped for upload). Interestingly, there is only one Q677P mutant (from 04 /2020, Spain) that shows up before November. October and November starts to show a few in TX, CA, VA, NM, FL, then in Dec and Jan just blows up in Louisiana as well as in TX, MA, NM, + + + , Would be nice to have a professional gen-epi person run some analyses on this, but it's curious to observe as long as we don't over-interpret it..

This Q677 is near the furin cleavage site (PRRAR 681-685). So it may be biologically interesting. Attaching a couple figures.. one is the phylogeny from our website.. but it does not include the new ones.

Can any of you model it into the Spike to predict if the "kink" of the Pro might change proteolytic processing? Image attached shows the rise of this mutation in our Louisiana build. .but this figure does not yet add our latest 56 new ones..

677-QTPTNSPRRAR-685
to
677-PTPTNSPRRAR-685
<image001.png>

From: Ikegame, Satoshi <satoshi.ikegame@mssm.edu>
Date: Wednesday, January 27, 2021 at 1:18 PM
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Cc: Mohammed Siddiquey <siddiquey27@gmail.com>, Lee, Benhur <benhur.lee@mssm.edu>, Brambilla, Luca (Student) <luca.brambilla@icahn.mssm.edu>, Ivanov, Stanimir S. <sivano@lsuhsc.edu>, Dragoi, Ana Maria <adrag1@lsuhsc.edu>, Kasopefoluwa Oguntuyo <sope.oguntuyo@icahn.mssm.edu>, Stevens, Christian (Student) <christian.stevens@icahn.mssm.edu>
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

We will be shipping B.1.351-GFP-VSV-CoV2 and VOC wt-GFP (18 tubes each).
Tracking number is 816009348652.

Titer of B1.1.7-GFP was 8*10^4 IU/ml (I made a mistake in calculation.)

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Wednesday, January 27, 2021 8:07:26 AM
To: Ikegame, Satoshi
Cc: Mohammed Siddiquey; Lee, Benhur; Brambilla, Luca (Student); Ivanov, Stanimir S.; Dragoi, Ana Maria; Kasopefoluwa Oguntuyo; Stevens, Christian (Student)
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Hi Satoshi,

1. That's fantastic if you can ship them today! Helps us move faster. I'll look at the preprint ASAP.

2. By the way, we just got 367 new CoV2 sequences in from late Dec - Jan 19. Not on GISAID yet — I have to process them for upload but many are:
P681H — shared with B.1.1.7
or
Q677H — allegedly shows up in new US variants of concern
or
Q677P

Here's an RTF file if any of you want to peruse it. I think we *may* have some new locally grown variants kicking around in the USA! https://www.dropbox.com/s/2bbzfgokbb8kwzd/muts_summ_20210127.rtf?dl=0

Summary of all mutations in our recent batch https://www.dropbox.com/s/l4hiyii3dwom6zr/myresults.csv?dl=0

Best,

Jeremy

Sent from phone (please forgive typos)

> On Jan 27, 2021, at 6:50 AM, Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:
>
> https://www.biorxiv.org/content/10.1101/2021.01.25.428137v2
> New study is released from Columbia university.
>
> Only 22 serum were tested, but quite similar to ours.
>
> I believe we can ship VOCwt-GFP (just collected and put into liquid nitrogen for quick freezing) and B1.358-GFP today.

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Ikegame, Satoshi
Sent: Tuesday, January 26, 2021 6:05:25 PM
To: Kamil, Jeremy P.
Cc: Mohammed Siddiquey; Lee, Benhur; Brambilla, Luca (Student); Ivanov, Stanimir S.; Dragoi, Ana Maria
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Dear Jeremy.

For growth assay.

1; I recommend to start from MOI=0.01 using 12 or 6 well plate.

2; I haven't checked growth in Vero or Vero-TM2 cells, but 72 hours must be enough to reach peak titer.

Replace half of medium twice a day up to 72 hours (every 24 hours check may fail to find peak because of fast growth).

3; I believe that we do not have to evaluate the cell associated viruses by scrape /freeze and thaw.

I believe that titer in supernatant will be fine unless special reason.

(To evaluate cell associated virus, we need sonicator. But I could not find good one for my measles virus project.)

4; For comparison of growth, I do not recommend to use different colored virus.

Sensitivity or brightness of FP may result in underestimation of titer (especially in E2-crimson) and make interpretation difficult.

For neutralizing assay by RBD-Fc;

I recommend to start from x 1/40 or 1/80 dilution (final concentration in the medium; supposed to be 145 or 72.5 ug/ml).

Then serial dilution of x1/4 (up to x1/4^7 = x1/16384).

GFP wont' be visible at > 10ug/ml.

(Actually we keep RBD-Fc in 4 degrees, but remains effective for several month.)

Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Tuesday, January 26, 2021 4:57:03 PM
To: Ikegame, Satoshi
Cc: Mohammed Siddiquey; Lee, Benhur; Brambilla, Luca (Student); Ivanov, Stanimir S.; Dragoi, Ana Maria
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Hi Satoshi and friends.. a few things (two are questions).

1. We got the B.1.1.7 GFP virus shipment. Thanks!

2. For the replication kinetics curves, please let us know your favorite approach or if we should just emulate what Sean Whelan or Kartik's paper's did (I'm assuming they did this)..
    a. What MOI should we use to infect the VERO-CCL81?
    b. What time points to take? I think a 48-72 h curve should be plenty even at low MOI.. but less if we go with higher MOI.
    c. We would -80 deg C freeze thaw 1X scaped cells to get cell associated + cell free titer all in one and obtain read out using TCID50.
    d. Idea is to make a curve for each of the mutants and wild-types in different color backgrounds.

3. We have C-T's Batch 5 RBD-Fc (in PBS), Conc: 2.9 µg/µL in ~ 500 µL, Total amount: 1450 µg. It is still in the original tube.
    a. If you have an ideal dilution series we should use on our assay plates as "internal standard" please let us know.
    b. My plan is to ask Siddiquey to make 20 aliquots of ~25 uL each. Or should I ask him to dilute it to ~1.5 mL of 1 ug/uL (1 mg/mL) in 25 mM HEPES Buffered Saline pH 7.5 + 10% glycerol + 0.05 mg/mL BSA. This way he could freeze back *snap freeze on liquid nitrogen—and

make ~30 aliquots with each being ~50 uL.

Cheers,

Jeremy

From: Ikegame, Satoshi <satoshi.ikegame@mssm.edu>
Date: Tuesday, January 26, 2021 at 8:18 AM
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Cc: Mohammed Siddiquey <siddiquey27@gmail.com>, Lee, Benhur <benhur.lee@mssm.edu>, Brambilla, Luca (Student) <luca.brambilla@icahn.mssm.edu>, Ivanov, Stanimir S. <sivano@lsuhsc.edu>, Dragoi, Ana Maria <adrag1@lsuhsc.edu>
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Dear Jeremy.

I just tittered B.1.1.7-GFP virus.

Titer was only 8x 10^3 IU/ml (Sample collection timing might have been a little bit earlier).

However, stock can make > 500 GFP events/well in 96 well at x1/10 dilution.

Sequence of genome RNA seems fine at least in spike-ORF.

B1.351-GFP has been collected this morning, and VOCwt-GFP will be collected tomorrow.

I believe we will ship both on next Monday.

Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Monday, January 25, 2021 7:03:13 PM
To: Ikegame, Satoshi
Cc: Mohammed Siddiquey; Lee, Benhur; Haas, Griffin (Student); Brambilla, Luca (Student); Ivanov, Stanimir S.; Dragoi, Ana Maria
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

This sounds really great, Satoshi. :-)

Sent from phone (please forgive typos)

> On Jan 25, 2021, at 5:31 PM, Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:
>
> I think mScarlet1 wild type is Florian's codon optimized sequence.
>
> So I will try to rescue VOC wild type-mScarlet1.
>
> (If we finish assay within 12hours (single round infection), codon selection won't be so problematic.)
>
> Satoshi Ikegame
>
> mail to:
>
> satoshi.ikegame@mssm.edu
>
> Department of Microbiology, Icahn School of Medicine at Mount Sinai.
>
> ---
>
> **From:** Kamil, Jeremy P. <jkamil@lsuhsc.edu>
> **Sent:** Monday, January 25, 2021 3:10:39 PM
> **To:** Ikegame, Satoshi
> **Cc:** Mohammed Siddiquey; Lee, Benhur; Haas, Griffin (Student); Brambilla, Luca (Student); Ivanov, Stanimir S.; Dragoi, Ana Maria
> **Subject:** Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

This is great news, Satoshi. I do think Stan likes to use mScarlet-I for wild type.

Siddiquey: please make sure we have plenty of mScarlet WT D614G Del21 grown up!

Best,

Jeremy

Sent from phone (please forgive typos)

> On Jan 25, 2021, at 2:03 PM, Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:
>
> We are about to ship B.1.1.7-GFP-VSV-CoV2 (I did not count number of tubes, but > 15 tubes).
>
> Tracking number is 816009348663.
>
> VOC wild type-GFP and B1.358-GFP are now being amplified, they will be ready by the end of week.
>
> We will also work on mScarlet-1 and E2-crimson later.
>
> But I slightly remember that Stan is planning to change mScarlet1 with other FP.
>
> Let me know if you are planning to use different colors other than mScarlet1.

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Sunday, January 24, 2021 9:49:14 PM
To: Ikegame, Satoshi
Cc: Mohammed Siddiquey; Lee, Benhur; Haas, Griffin (Student); Brambilla, Luca (Student)
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Look forward to reading this. Reminds me of findings from the paper from Vineet Menachery (Benhur is a coauthor too)..

I wonder if there isn't also an enhancement of cell free infectivity when polybasic site is mutated?!

IMO worth looking at, at some point...

Sent from phone (please forgive typos)

On Jan 24, 2021, at 6:17 PM, Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:

https://www.biorxiv.org/content/10.1101/2021.01.22.427802v1

This paper may be interesting for you.
Cleavage site mutations happen without protease expression, and increase entry into VeroE6 cells.

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Sunday, January 24, 2021 6:29:30 PM
To: Ikegame, Satoshi; Mohammed Siddiquey
Cc: Lee, Benhur; Haas, Griffin (Student); Brambilla, Luca (Student)
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Hi Satoshi,

Thanks for your update, too. .that is super exciting. Based on our observation that the only mutation we saw in Sean Whelan’s virus was the PRRAR à PRRAS mutation, I doubt you would detect any mutations arising quickly in the freshly recovered VSV-B.1.1.7 Spike but never hurts to have the data (in case reviewers ask for it.)

In any case, we are excited to test it here for escape from neutralization!

Best,

Jeremy

From: Satoshi Ikegame <satoshi.ikegame@mssm.edu>
Date: Sunday, January 24, 2021 at 5:26 PM
To: Jeremy Kamil <jkamil@lsuhsc.edu>, Mohammed Siddiquey <siddiquey27@gmail.com>
Cc: Benhur Lee <benhur.lee@mssm.edu>, Griffin Haas <griffin.haas@icahn.mssm.edu>, "Brambilla, Luca (Student)" <luca.brambilla@icahn.mssm.edu>
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Dear Jeremy.

Thank you for your update.

I just collected B.1.1.7 VSV-CoV2GFP.

I will ship tomorrow or on Tuesday approx 20 tubes.

Luca will work on sequencing genome RNA to see if no mutation happens in spike gene.

This will be done in several days, and will let you know result.

Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Sunday, January 24, 2021 1:54:25 PM
To: Mohammed Siddiquey; Ikegame, Satoshi
Cc: Lee, Benhur; Haas, Griffin (Student)
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Hi Satoshi, (**Siddiquey**: please add this info to the glycerol stocks in the Google Drive).

Based on the latest data that came in from genewiz re-runs, we can now say these two also look good:

- pEMC-VSV-mScarlet full 501Y.V2 / B.1.351 # clone 1
- pEMC-VSV-eGFP_501Y.V2 (S African) RBD # clone 2

To provide a re-cap / summary including all the clones that Siddiquey shipped which appear to be good..

1. pEMC-VSV-eGFP_501Y.V2 (S African) RBD # clone 1
    2. pEMC-VSV-eGFP_501Y.V2 (S African) RBD # clone 2

Note the above two are RBD only “trifecta” of K417N/E484K /N501Y.. and will not need to use VOC_CTRL as a control.

3. pEMC-VSV-eGFP FULL 501Y.V2 (S African) # clone 2. This is FULL length clone.. will need VOC_CTRL for best data.
    a. ** I can’t read the very end of the gBlock because the Rev primer failed. but it is probably fine!
4. pEMC-VSV-mScarlet full 501Y.V2 / B.1.351 # clone 1
5. pEMC-VSV-eGFP VOC_CTRL # clone 2
    a. note, SILENT wobble mutation of 3rd position of Ser477 (TCT to TCC).. but no expected effect…
6. pEMC-VSV-mScarlet VOC_CTRL # clone 3, looks perfect!

Please let me know if you’d like me to email you the sequence files or alignments.

Cheers,

Jeremy

From: Jeremy Kamil <jkamil@lsuhsc.edu>
Date: Friday, January 22, 2021 at 1:47 PM
To: Mohammed Siddiquey <siddiquey27@gmail.com>, Satoshi Ikegame <satoshi.ikegame@mssm.edu>
Cc: Benhur Lee <benhur.lee@mssm.edu>, Griffin Haas <griffin.haas@icahn.mssm.edu>
Subject: RESULTS B.1.351 and VOC_CTRL

Sequencing Summary

Hi Satoshi,

Of the plasmids and bacteria that Siddiquey shipped on Tuesday, here’s our results from Genewiz..

These clones look **GOOD or LIKELY GOOD** by Sanger Sequencing.

1. pEMC-VSV-eGFP_501Y.V2 (S African) RBD # clone 1 **#RBD only!** This one will not need to use VOC_CTRL as a control.
    2. pEMC-VSV-eGFP FULL 501Y.V2 (S African) # clone 2. This is FULL length clone.. will need VOC_CTRL for best data.
        a. ** I can’t read the very end of the gBlock because the Rev primer failed. but it is probably fine!
    3. pEMC-VSV-eGFP VOC_CTRL # clone 2
        a. note, SILENT wobble mutation of 3rd position of Ser477 (TCT to TCC).. both codons pretty much equally abundant.. no expected effect!
    4. pEMC-VSV-mScarlet VOC_CTRL # clone 3, looks perfect!

These clones are **BAD:**

5. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 2.
    a. Missense mutation of V610I (numbering per Wuhan Hu-1 REF Spike) …this would be Val606Ile in B.1.351 (501Y.V2) Spike
    b. Silent unintended GGC to GGT wobble mutation at Gly codon 252 (Wuhan REF numbering).. this would be Gly249 of actual 501Y2 translation product.

These clones I can’t tell yet..

1. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 1
    a. can’t read ATG at 5’-end due to smear.
2. Some others didn’t sequence well yet either..

Here is the raw data https://www.dropbox.com/s/2ebdfnpm42rkfgv/JEREMYKAMIL01-22-2021_182552_347.zip?dl=0

And the alignments: https://www.dropbox.com/sh/27uz4816j3xolll/AACc5_7RMFJ8jP_nT4aFAgfsa?dl=0 (some are macvector format but also FASTA are provided).

Cheers,

Jeremy

From: Mohammed Siddiquey <siddiquey27@gmail.com>
Date: Tuesday, January 19, 2021 at 7:06 PM
To: Ikegame, Satoshi <satoshi.ikegame@mssm.edu>
Cc: Lee, Benhur <benhur.lee@mssm.edu>, Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Subject: Re: SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor | bioRxiv

EXTERNAL EMAIL: EVALUATE

Hi Satoshi,

I am sending you the following today. The tracking number is 772672573621.

Please let me know after receiving.

Thank you.

Siddiquey

DNA (20 ul, >100 ng /ul):

1. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 1
2. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 2
3. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 3

4. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 1
5. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 2
6. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 3

7. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 1
8. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 2
9. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 3

10. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 1
11. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 2

12. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 3

13. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 1

14. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 2

15. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 3

16. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 1

17. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 2

18. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 3

19. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 1

20. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 2

21. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 3

Bacteria (50 ul):

22. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 1

23. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 2

24. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 3

25. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 1

26. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 2

27. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 3

28. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 1

29. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 2

30. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 3

31. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 1

32. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 2

33. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 3

34. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 1

35. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 2

36. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 3

37. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 1

38. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 2

39. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 3

40. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 1

41. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 2

42. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 3

On Thu, Jan 14, 2021 at 1:49 PM Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:

> I noticed I cannot start transformation until you get sequence result.
>
> Sorry for the confusion.

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Ikegame, Satoshi
Sent: Thursday, January 14, 2021 2:46:44 PM
To: Mohammed Siddiquey; Lee, Benhur
Cc: Kamil, Jeremy P.
Subject: Re: SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor | bioRxiv

Hi Shiddiquey.

I just received package and will start transformation.

Thank you.

Next time, could you send us bacteria (20ul LB medium in tube) in addition to plasmid if possible?

Best,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Mohammed Siddiquey <siddiquey27@gmail.com>
Sent: Wednesday, January 13, 2021 8:46:14 PM
To: Lee, Benhur
Cc: Ikegame, Satoshi; Kamil, Jeremy P.
Subject: Re: SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor | bioRxiv

Hi Satoshi,

I have sent you the following gBlocks and DNA today. Tracking number is 772622125267.

Please let me know after receiving. I am retransforming 417_501 NKY mutant. Colonies from my first transformation are a little weird. Hopefully I can send you some good clones soon.

Thank you.

Siddiquey

gBlocks:

1. Bsu Sph 417_501 NKY (gBlocks, 10 ng/ul), 20 ul
2. B.1.1.7 (gBlock 1, 10 ng/ul) 20 ul
3. B.1.1.7 (gBlock 2, 10 ng/ul) 20 ul

DNA (20 ul, 100 ng /ul):

4. pEMC-VSV-eGFP D614G_B.1.1.7 # clone 1
5. pEMC-VSV-eGFP D614G_B.1.1.7 # clone 2
6. pEMC-VSV-eGFP D614G_B.1.1.7 # clone 3
7. pEMC-VSV-eGFP D614G_B.1.1.7 # clone 4
8. pEMC-VSV-mScarlet D614G_B.1.1.7 # clone 1
9. pEMC-VSV-mScarlet D614G_B.1.1.7 # clone 2
10. pEMC-VSV-mScarlet D614G_B.1.1.7 # clone 3
11. pEMC-VSV-mScarlet D614G_B.1.1.7 # clone 4
12. pEMC-VSV-E2_Crimson D614G_B.1.1.7 # clone 1
13. pEMC-VSV-E2_Crimson D614G_B.1.1.7 # clone 2

On Mon, Jan 11, 2021 at 5:33 PM Lee, Benhur <benhur.lee@mssm.edu> wrote:

> Hi Jeremy,
>
> I got two random cold calls (emails) today asking me

if I have the 501.V2 variant: N501Y, K417N, E484K? One was from a colleague with VA connections saying they want to test 55 COVID-19 positive sera.

What the hell are people thinking? That you (we) make & rescue them just for fun so others can test them? (How do they know we're even doing this?)

My answer is going to be "No, but my collaborators *may* have made them and they are testing them on their panel of sera".

Sounds good? We better get this out soon. Meanwhile, I just asked Satoshi to start virus evolution expt using sera on our rcVSV-CoV2-S. Since we already have the individual N501Y, delta-F140, and E484K *individual* mutants, do you think starting from one of these mutants will be faster?

(**Satoshi, attached is the paper I was talking to you about earlier . . . it took them 5-8 days for each passage and 7 passages (45 days) to get their first mutant and 14 passages (85-95 days) to get a completely resistant virus: delta F140, E484K, 248-KTRNKSTSRRE 11aa insertion. I think we can do each passage at 24-36 hrs and get resistant mutants in 2 weeks, especially since VSV mutates faster than SARS-CoV-2).**

Anyway, seems like everyone is rushing this, no more being number two after all the hard work we all have put into making this system work. BTW, I find it impossible that a 11-aa insertion spontaneously occurs. Don' you think it must be present a very rare frequency or some sort of recombination event occurred?

Should we have a joint call to determine priorities once your study section is over?

Benhur

Benhur Lee, M.D.
Professor of Microbiology
Ward-Coleman Chair in Microbiology
Icahn School of Medicine at Mount Sinai
One Gustave L Levy Place #1124
New York, NY 10029
Office: (212) 241-2552 | Lab: (212) 241-9960

Lab Webpage: LeeLabVirus.Host **| Twitter** @VirusWhisperer

> On Jan 11, 2021, at 2:56 PM, Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:
>
> N501Y, N501Y+E484K have higher affinity.
>
> This cannot be found in my VSV-CoV2spike.
>
> We may need to condider different kind of assay to evaluate S vs ACE2 affinity.
>
> It is good to know that you've already cloned 501V1 and 501V2.
>
> Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Monday, January 11, 2021 2:44:40 PM
To: Lee, Benhur; Haas, Griffin (Student); Ikegame, Satoshi; Mohammed Siddiquey; Hung, Chuan-tien; Dragoi, Ana Maria
Subject: SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor | bioRxiv

USE CAUTION: External Message.

https://www.biorxiv.org/content/10.1101/2021.01.06.425392v2

https://twitter.com/_b_meyer/status/1348695525483290625?s=21

“ While the single 501Y mutations causes a ~2.5x higher affinity to Ace2, the double mutation with E484K results in a ~13x higher affinity to Ace2 compared to the WT version.”

<image0.jpeg>

We have this cloned already (as RBD) of S Africa variant B.1.351, in the GFP VSV Spike backbone.

Cheers,

Jeremy

Sent from phone (please forgive typos)

From: Kevil, Christopher <CKevil@lsuhsc.edu>
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Dan Snyder <dan@migscenter.com>
Date: 1/27/2021 12:32:42 PM
Subject: Re: Sequencing

Right Jeremy. Great points all around.

One question I have for Dan and Vaughn, have you guys seen the UK variant in Pitt or in sequences from other regions? If so, how prevalent are they?

It seems to me that the 'UK variant' itself would change over time and host thus making it difficult to really say 'yes or no' where it has been.

I agree with Jeremy that there are US variants in the mix challenging spread of other variants.

Best,

Chris

From: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
Date: Wednesday, January 27, 2021 at 5:51 AM
To: Dan Snyder <dan@migscenter.com>
Cc: Vaughn Cooper <vaughn.cooper@pitt.edu>, Chris <CKevil@lsuhsc.edu>
Subject: Sequencing

Hi Dan,

Looping in our Vice chancellor, Chris Kevil, here. I think everyone agrees that the Hyb Enrichment Seq you guys are doing is excellent / gives such nice results.   I'm not sure when / if we may like to try out an ARTIC protocol, but assuming y'all might offer it down the road, please let us know about pricing and availability.

1.   It sounds like La Dept of Health might come be able to support some of our sequencing going forward.. maybe only about 200 sequences per month?   Dr. Kevil will know more re the details.

2.   In CONFIDENCE — preliminary analyses (Pangolin and Spike aa mutation scanning) suggest there may be a few interesting "variants" in the latest 367 sequences you just finished and uploaded to Box. I'm seeing ones with Spike P681H, Q677H, and Q677P, alongside unique constellations of other clade / lineage defining polymorphisms. The first two of those amino acid changes in Spike are already implicated as being "of concern" since these are close to the furin cleavage site aka "polybasic" PRRAR motif. However, I did not see any classic B.1.1.7 / 501.Y2 "UK" variant.. yet.   (The UK variant does have P681H.. the COG-UK team call that mutation "Pooh" like as in "Winnie the Pooh," similarly they call D614G, "Doug", and N501Y "Nelly").

My suspicion is that there could just as well be USA homegrown variants could be competing fine against the B.1.1.7. Perhaps explaining why it hasn't begun to take over yet?   Shreveport might not be NYC or Los Angeles, but we are along a major travel corridor (I-20 / I-49) so I think we are well poised to pick it up.   I'm just glad we are NOT explicitly pre-screening for B.1.1.7 by S amplicon drop out RT-qPCR because that IMO is garbage for Gen-Epi (adds a huge bias and removes ability to detect homegrown variants that might be out competing others).

Anyway, I'll paste in the "PS" section below some Dropbox links to the csv outputs of some crude web tool analyses I ran last night on the data, in case anyone wants a quick peek. At some point, I'm gonna have to start writing like a half dozen manuscripts, and of course you and Vaughn are on all of them.

Cheers,

Jeremy

PS:

https://www.dropbox.com/s/2bbzfgokbb8kwzd/muts_summ_20210127.rtf?dl=0

https://www.dropbox.com/s/5sg7dg8swq806q4/results_Pangolin_COG_UK_20210127.csv?dl=0

https://www.dropbox.com/s/l4hiyii3dwom6zr/myresults.csv?dl=0

Sent from phone (please forgive typos)

From: Lee, Benhur <benhur.lee@mssm.edu>
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Date: 10/21/2020 6:48:02 PM
Subject: Re: Plates of RNA samples..

I'm obviously out of the loop . . . had food poisoning on Sunday-Mon, then had tio gte cleared to return to work since diarhhea is one of the symptoms but keith and I could trace it back to a single source of eating expired food. Sigh.

Anyway, I'm not sure what these samples are, or if I am mean to do something.

Benhur

Benhur Lee, M.D.
Professor of Microbiology
Ward-Coleman Chair in Microbiology
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Office: (212) 241-2552 | Lab: (212) 241-9960

Lab Webpage: LeeLabVirus.Host **| Twitter** @VirusWhisperer

> On Oct 16, 2020, at 1:41 PM, Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:
>
> USE CAUTION: External Message.
>
> Hi Dan, (also copying Vaughn and my superb collaborator Benhur Lee so they can have this info, too)
>
> This is great news! Now I'll check if I can find a BSL-3 CoV2 lab that might be willing to transfect them. Attaching a file with the plate and well info + a .gb file that annotates the reference sequence with the mutations.
>
> ONE IDEA.. if we do find a BSL3 collaborator to transfect them, we can even pool together each set of samples that has an identical virus in it, which might help increase success of recovery..
>
> My friend Nat Moorman (Assoc Prof at UNC CH) is in same Dept and collaborates with Ralph Baric and Mark Heise.. They might help us look at some of these in a mouse model… Though he suggested that the UNC folks can just put the mutations into a mouse-adapted infectious clone.
>
> EVTL607 and 614 are identical to each other, so that's like 10-16 uL..
> EVTL 410-417 (8 viruses) are identical to each other… so that gives at least 40 uL
> EVTL 437-438 are identical too, so that's like 10-16 uL..
>
> Crossing fingers that we can find someone to rescue these.. (assuming that I'm right that this is worth doing .. haha).. seems like a good insurance policy to maximize impact of any epi-papers re these viruses the outbreaks we found them from…
>
> Best,
>
> Jeremy
>
> ---
>
> From: Dan Snyder <dan@migscenter.com>

Date: Friday, October 16, 2020 at 12:01 PM
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Subject: Re: Plates of RNA samples..

EXTERNAL EMAIL: EVALUATE
Hi Jeremy,

We do still have the original RNA plates, which were immediately frozen again after we pulled our aliquot. I would guess we have around 5-8uL or RNA left in each well.

Dan

On Fri, Oct 16, 2020 at 1:36 AM Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:

> Hi Dan,
>
> There are about 12 samples from the most recent set of 4 plates you guys just sequenced from that are potentially worth growing up. Found out that our testing lab tossed out all the remaining original viral transport media samples corresponding to those from the -80 freezers.
>
> Just wondering if you guys still have any of the RNA left ... in case I can find a BSL-3 CoV2 collaborator who wants to try to transfect it into VERO cells.
>
> Long shot.. but just curious. (I'm furious that our testing lab threw the original swab + VTM samples away.. Argh!)
>
> Cheers,
>
> Jeremy
>
> Sent from phone (please forgive typos)

--
Dan Snyder, Director
Microbial Genome Sequencing Center
355 Fifth Avenue, Suite 810
Pittsburgh, PA 15222
(412) 475-2318

<Coded Facility name_mutations_20201015 ORF7a Spike etc.xlsx><EPI_ISL_402124 WIV4.gb>

From: Vanchiere, John A. <jvanch@lsuhsc.edu>
To: Yurochko, Andrew D <AYuroc@lsuhsc.edu>
Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sapp, Martin J <MSapp1@lsuhsc.edu>
Scott, Rona S <RScott1@lsuhsc.edu>
Date: 9/1/2020 1:20:32 PM
Subject: Preprint from UTMB on SARS-CoV-2 Pathogenesis
Attachments: Furin_Cleavage_Site_Is_Key_to_SARS-CoV-2_Pathogene (1).pdf

---

John A. Vanchiere, MD, PhD
Professor of Pediatrics
LSUHSC-Shreveport

p-318-675-7877
f-318-675-6059

From: Steven Meyers <steven@gisaid.org>
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Date: 6/26/2020 12:40:37 AM
Subject: Re: Correction to covv_add_location for 3 nCoV-19 EPI_ISL

It is and never has been about collaboration for these kind of individuals. They wouldn't know how to spell it.

https://ncbiinsights.ncbi.nlm.nih.gov/2020/06/25/new-genbank-submission-options-sars-cov-2/

their deep rooted anger is plainly visible and ironically not a product we can put in the box of this admin or political ideology

---

From: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
Date: Thursday, June 25, 2020 at 15:17
To: GISAID EpiCoV Team <hCoV-19@gisaid.org>
Cc: Steven Meyers <steven@gisaid.org>
Subject: Re: Correction to covv_add_location for 3 nCoV-19 EPI_ISL

Hello EpiCov Team,

(1) Firstly, Thanks! You guys are great.

(2) Secondly, I found a few more EPI_ISL's that had Sex / gender data on the patient missing... here are the sex / gender for a list of Accessions that I think are currently missing those metadata.

EPI_ISL_435555. Sex = Female
EPI_ISL_435557. Sex = Female
EPI_ISL_435558. Sex = Female
EPI_ISL_435560. Sex = Female
EPI_ISL_435561. Sex = Female
EPI_ISL_435562. Sex = Female
EPI_ISL_435563. Sex = Female
EPI_ISL_435564. Sex = Female

(3) Third, thanks for the heads up about the "Additional Location Field". For future uploads I will enter "Parish name" into the main location field. Thanks for updating those records with the Parish name in the Location field instead of the "additional location field"

(4) I am proud to support GISAID on Social Media.. I sincerely believe in the importance of your work. (It's easy to see how much you guys make a difference!!!). I've also been telling a few different journalists (when they call me) about how important GISAID is, and that it's a great story to tell the public about how open data helps us react to pandemics, and prepare vaccines quickly, etc.. how NIH / NCBI failed to solve the incentive problem raised by Cox and Bogner in that Nature paper back in August of 2006! Sadly, I still don't think USA is trying seriously to solve that problem and I feel like CDC is perhaps trying to "compete" with GISAID instead of cooperate.. which of course would be the better idea... So far spoken to folks from Washington Post, USA Today, and Wired.

(5) I'm of course not at all affiliated with GISAID.. but it kind of pissed me off that the authors of this Nature Medicine paper hardly mention GISAID.. https://www.nature.com/articles/s41591-020-0935-z

Am I misunderstanding something? Look at Figure 1.. it shows big boxes labeled NCBI, ENA, & Pathogenwatch but not GISAID.. the only place you see GISAID is in the legend to fig 1, as a parenthetical "(e.g. GISAID, ViPR)"

> At same time, I have a developed a decent rapport with Duncan MacCannell (CDC) – he reached out to me a month or so back, and invited me to join his “CDC Spheres” Slack group.. he is supportive of our team getting some CDC funding to continue sequencing in NW Louisiana.. so I have to be diplomatic when / if I publicly object to this type of stuff if it involves him. On the plus side, I happen to know Duncan seems to be aware that the CDC has “messed up” (egg on their face, big time) but the impression I get (not subtle) is that their / his hands are tied by the highest levels of US Govt (Political Appointees). Similarly, as you probably saw, EcoHealth Alliance / Peter Daszak had their funding for bat coronavirus work in China pulled because of political interference here (disgusting!) and Francis Collins (NIH Director) was powerless to do stop this travesty from happening.. probably because he’d lose his job.

Have a nice evening!

Best,

Jeremy

From: GISAID EpiCoV Team <hCoV-19@gisaid.org>
Date: Thursday, June 25, 2020 at 3:38 PM
To: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
Cc: GISAID EpiCoV Team <hCoV-19@gisaid.org>, Steven Meyers <steven@gisaid.org>
Subject: Re: Correction to covv_add_location for 3 nCoV-19 EPI_ISL

Dear Kamil,

Many thanks for the opportunity to update your records. We noticed on these three cases that you are using the Additional location field. The idea of this field is not to narrow down the geolocation, but to express a characteristic such as a concert hall, a football stadium, or a wholesale market like we have recently seen in Beijing.

We have updated the Location accordingly and hope you are in agreement

Many thanks for your steadfast support of your platform on social media we have recently learned about.

Best wishes

Your GISAID EpiCoV Team

P.S.: Be sure to email us directly with your data needs to this **hcov-19@gisaid** email address. It is dedicated to support Submitters with priority

---

From: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
Date: Thursday, June 25, 2020 at 13:10
To: GISAID EpiFlu Database Support <service@gisaid.org>, Steven Meyers <steven@gisaid.org>
Subject: Correction to covv_add_location for 3 nCoV-19 EPI_ISL

Hi Steven and GISAID Support Team,

Would you please help me fix an element of metadata for 3 of our uploaded nCoV-19 sequences?

We accidentally populated Incorrect Data for the "covv_add_location" field when we bulk uploaded the nCoV19 sequences for the following EPI_ISL accessions:

EPI_ISL_451274
EPI_ISL_451282
EPI_ISL_451292

The CORRECT value for "covv_add_location" field for all three of these EPI ISL Accession numbers is "Bossier Parish"

At the time of upload, they were incorrectly labeled as "Caddo Parish" due to a data entry error on our end. That has now been rectified.

Apologies for the error and thank you for your help!

Best,

Jeremy

On 6/1/20, 5:02 PM, "GISAID Support Team" <service@gisaid.org> wrote:

EXTERNAL EMAIL: EVALUATE

Dear "Kamil, Jeremy P." <jkamil@lsuhsc.edu>,

Thank you for contacting GISAID support. We will try and respond to all messages within 1 business day. If you are contacting us with a technical question, kindly allow more response time, as we may need to consult with our technical staff.

Kind Regards,

Your GISAID Support Team

From: Mouton, Drew <dmout6@lsuhsc.edu>
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Date: 6/12/2020 9:39:32 PM
Subject: Re: Article: Mouse model of COVID-19

Yeah, one of the things I recognize we're fighting (at least a little bit) is the drive towards "fancy" science and elegant solutions, versus things that work well enough to accomplish the need.

Perhaps the AD transduction or Baric transmuting the virus is the latter. That said, this is the rest of what Xiaohong said (and thinks as to why this is important);

"In fact, this [the Cell paper] is a very impetuous model, worse than Zhengli Shi's transgenic mice using lung cell-specific promoters also published at Cell. All these mice cannot be used to model the human infection and the complications of COVID-19 because of the promoter they used. We need a transgenic mouse model of ACE2 with full-length human gene regulatory region, not only to be able to be infected by the SARS-CoV-2 but also for the infection process to be as human-like as possible. A lot of companies are rushing to generate Ace2 animal models. I have called all these companies, none of them using the full-length human promoter as I declared, largely due to the technical challenge."

But if it takes him 5 months I expect others will be ahead of the game. Unless we can find a way to speed it up.

D

> On Jun 12, 2020, at 3:50 AM, Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:
>
> Hi Drew,
>
> Agree with your takes! Do we need another recombinant mouse with human ACE2? Could be valuable and Xiaohong Lu can probably make one. It will take a while and others are doing it. Not bad idea but only worth something if it's gonna be used in experiments... many of which will already be published by the time his mouse is ready. Overall hard to say whether it's worth doing but on its face it's a useful tool to have. Just can't say —genuinely uncertain—whether cost benefit pans out.
>
> That cell paper takes a shortcut. Pretty slick. Adenovirus (Ad) vector transduction of human ACE2 into mice. Making them express human ACE2 without having to create a new mouse line. Basically Ad vectors do a great job carrying genes into whatever cells we need them too.
>
> Ralph Baric's lab meanwhile mutated the SARS-CoV-2 Spike at 2 amino acids so that the virus could use mouse ACE2 for entry. Thereby opening up mice as a model by making that tiny change to the virus (instead of the mouse). That paper came out about a month ago. Slam dunk approach!
> https://www.biorxiv.org/content/10.1101/2020.05.06.081497v1

Drew Mouton
Chief Innovation Officer
Office of Research
LSU Health Shreveport
318-675-7016 phone
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dmout6@lsuhsc.edu

Confidentiality notice: privileged/confidential information may be contained in this message. ***This correspondence is covered by the electronic communications privacy act, 18 u.s.c. § 2510-2521 and is legally privileged.***

From: Courtlin Vinson <courtlinv8@gmail.com>
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Date: 6/10/2020 5:01:55 AM
Subject: Re: Virology related jobs in NYC

EXTERNAL EMAIL: EVALUATE
Hi Jeremy,

Sorry for the late reply, I will definitely check them out, and be sure to apply. Thank you so much for letting me know about this opportunity!

Respectfully,

Courtlin D. Vinson

On Tue, Jun 9, 2020 at 11:09 PM Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:

> This (EcoHealth Alliance) is a fantastic organization.   You should apply!   They do work related to preventing pandemics and viral outbreaks and they have openings right now:
>
> https://www.ecohealthalliance.org/careers
>
> Here’s a tweet about it
>
> https://twitter.com/ecohealthnyc/status/1270004832359809025?s=21
>
> Sent from phone (please forgive typos)

From: Lee, Benhur <benhur.lee@mssm.edu>
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Date: 6/8/2020 6:02:21 PM
Subject: Re: Quick update re RBD mutants, PBMC, etc

just s sec. sett8jng it up.

#BeSafe #FlattenTheCurve #SocialDistancingWorks

Benhur Lee, M.D.
Professor of Microbiology
Ward-Coleman Chair in Microbiology
Icahn School of Medicine at Mount Sinai
One Gustave L Levy Place #1124
New York, NY 10029
Office: (212) 241-2552 | Lab: (212) 241-9960

Lab Webpage: LeeLabVirus.Host **| Twitter** @VirusWhisperer

> On Jun 8, 2020, at 2:01 PM, Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:
>
> YES
>
> From: "Lee, Benhur" <benhur.lee@mssm.edu>
> Date: Monday, June 8, 2020 at 1:00 PM
> To: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
> Subject: Re: Quick update re RBD mutants, PBMC, etc
>
> you’re free for a quck zoom? benhur
>
> #BeSafe #FlattenTheCurve #SocialDistancingWorks
>
> Benhur Lee, M.D.
> Professor of Microbiology
> Ward-Coleman Chair in Microbiology
> Icahn School of Medicine at Mount Sinai
> One Gustave L Levy Place #1124
> New York, NY 10029
> Office: (212) 241-2552 | Lab: (212) 241-9960
>
> **Lab Webpage:** LeeLabVirus.Host **| Twitter** @VirusWhisperer
>
>> On Jun 8, 2020, at 1:23 PM, Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:
>>
>> Hey Benhur,
>>
>> A few quick things/ questions:
>>
>> 1. For the panel of RBD mutants, would you recommend FOR or AGAINST truncating the cytoplasmic tail of Spike in pCAGGS? Sean Whelan’s VSV SARS2 Spike paper seemed to show a great growth advantage after it spontaneously truncated the last 21 aa. (Deletion of 1253-1273). But re-reading some of your other emails it seems like maybe that’s not a good plan? I’m confused!
>>
>> 2. Just so you know… I didn’t spill the beans to my friend Vic DeFilippis (OHSU) about all the details of your nanopore partnership, or your elegant FACS methods…. just told him that you had a hook up for free scRNA sequencing of B-cells from SARS2 seropostives. Turns out he’s friendly with Bill Messer (a

2. Ralph Baric trainee) who is banking lots of PBMC at OHSU.. in case that's helpful to you he can set up an intro. Anyway, Vic is an ally and friend who I got to know via one of my very best buddies, Chris Benedict (a viral immunologist at La Jolla Institute). So, I think you can trust him in case the connection might be helpful to you.

3. I got all the wheels turning here toward getting PBMC collection authorized / IRB approved. Even got a "buy-in" from a physician on our IRB board named Connie Arnold here who helps lead outreach / free SARS-CoV-2 testing for underserved communities (FQHCs). Basically, we have three major sources of blood donor: (i) staff at the hospital who have already seroconverted, (ii) the FQHCs / community health centers we serve, and (iii) the convalescent sera donation pipeline via "LifeShare." It will be a bit of IRB paperwork but I'm happy to do that.. it will probably take a couple weeks minimum to get all the approvals in place.. and we will need a DUA with Mt. Sinai, which can also be expedited. If it helps you, I'm happy to lead the charge and start writing IRB protocol! Connie told me she can help me with the consent forms. We can start with BAMBANKER freezing PBMC to ship to. you. But I also have 10XGenomics sales team in my inbox. .so can pursue that independently, too.

Hope you had a good weekend!

Cheers,

Jeremy

From: "Lee, Benhur" <benhur.lee@mssm.edu>
Date: Tuesday, June 2, 2020 at 6:33 PM
To: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
Subject: Re: Spike RBD mutants...

EXTERNAL EMAIL: EVALUATE
GO ahead and order, especially since cloning is your middle name. By the time you have those made,we can decide to either rescue all of them as pseudotypes (since we use Florian's pCAGS gene, it will be perfect), or as replication competent clones in VSV. I'm on the IBC and DURC, this does NOT violate any "gain-of-funtion" studies because, they are ,by definition, naturally occurring mutations.

Turns out that growth of *live* SARS-CoV-2 in 293T-ACE2/TMPRSS2 double stables is about 10-fold higher than anything we have seen in the past, so this is likely something our department will switch to using. If I can rescue full-length Spike without tail truncation on VSV using these 293T-ACE2/TMPRSS2 cells, that would make me very very happy. We should send you these cells to do the your future VNAs; we think we might finally have the stable Vero-ACE2/TMPRSS2 as well, we'll see.

BTW, you should get a final draft of our paper to review tomorrow or Thursday. We intend to upload onto BioRxiv on this weekend.

And no, FBI won't come calling, I've ordered sequences that span almost the entire Nipah virus genome and no one cares.

Benhur

#BeSafe #FlattenTheCurve #SocialDistancingWorks

Benhur Lee, M.D.
Professor of Microbiology
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One Gustave L Levy Place #1124
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Lab Webpage: LeeLabVirus.Host | **Twitter** @VirusWhisperer

On Jun 2, 2020, at 5:38 PM, Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:

USE CAUTION: External Message.

Hi Benhur,

So, here’s a couple of exports from UCSF Chimera.. turns out all the GISAID RBD mutants that show up 2 or more times are between aa 439-494. Pretty small patch of the RBD.. only 56 aa. And… even though there are 23 mutations found so far.. only 12 show up 2 or more times, and only 6 show up 5 or more times..

WT sequence is NNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQS

The attached Chimera images show this 439-494 patch on the RBD “up,” and another in on an RBD “down” subunit.

Anyway, if you use Florian’s pCAGGS-Spike to pseudotype, there’s an MluI – SphI release-able fragment that could be replaced by IDT gBlocks of 517 bp, even including 20 bp Gibson flanks for assembly.. I already checked and this fragment’s sequence passes their “complexity” filters.. though probably the FBI will visit me once I place an order.

Cheers,

Jeremy

Mutation	**Occurence**
N439K	154x: UK, Romania
T478I	52x: England
V483A	30x; USA, Englad
G476S	10x; USA, Belgium
S494P	7x; USA, UK, EU, India,
V483F	5x; Spain, UK
A475V	4x; USA, Australia
G446V	3x; Australia, UK
V483I	2x; UK
F490L	2x; Austrialia, USA
F490S	2x; UK
Q493L	2x; USA

<imageSpike RBDdown_439_434.png><imageSpike RBDup_439_434.png>

From: Yurochko, Andrew D <AYuroc@lsuhsc.edu>
To: Orr, Anthony W. <aorr@lsuhsc.edu>
Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Kevil, Christopher <CKevil@lsuhsc.edu>
Woolard, Matthew D. <mwoola@lsuhsc.edu>
Sapp, Martin J <MSapp1@lsuhsc.edu>
Date: 5/8/2020 1:29:13 PM
Subject: Re: The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice | Nature

---

Sorry,
I forgot to send to all. I sent to Chris. The CEAR can do 60K and the grant has to be on an immunology-related topic. If the money is mixed with say the FWCC money for a larger grant, the grant would have to be on Immunology-related topic. Ed also checked with Jeff Reynolds about the use of the money. And as you can imagine the carryover was a fun budget discussion.
-A

Andrew D. Yurochko, Ph.D.,
Professor and Carroll Feist Endowed Chair in Viral Oncology,
Vice Chair, Dept. of Microbiology & Immunology,
Dir. of Research, Center of Excellence in Arthritis and Rheumatology,
Dir. of Research, Feist-Weiller Cancer Center,
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1501 Kings Highway,
Shreveport, LA 71130-3932.
Phone: 318-675-8332
Fax: 318-675-5764

On 5/8/20, 8:05 AM, "Orr, Anthony W." <aorr@lsuhsc.edu> wrote:

See the attached preprint that Jeremy Kamil sent me this morning describing a mouse-adapted SARS-CoV-2 produced in the lab of Dr. Ralph Baric at UNC - Chapel Hill. This model would be far superior to the hACE2 transgenic mouse model, since the ACE2 receptor will be expressed in the right cells and at physiologically relevant levels. While this paper does not go beyond 7 days post-infection (probably to ensure rapid publication), it may (or may not) provide insight into the cardiovascular complications of COVID-19 infection. So my questions are:

1. Is this something we want to pursue?
2. Does anyone know Ralph Baric to potentially reach out for the virus?

Wayne

A. Wayne Orr, Ph.D.
Director, Center for Cardiovascular Diseases and Sciences
Professor and Director, Division of Research
Department of Pathology and Translational Pathobiology
Office: 318.675.5462
Lab: 318.675.5463

-----Original Message-----
From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Thursday, May 07, 2020 3:25 PM
To: Alexander, Jonathan Steven <JAlexa@lsuhsc.edu>; Kevil, Christopher <CKevil@lsuhsc.edu>; Orr, Anthony W. <aorr@lsuhsc.edu>
Subject: The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice | Nature

https://nam01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.nature.com%2Farticles%2Fs41586-020-2312-y&amp;data=02%7C01%7Cjkamil%40lsuhsc.edu%7C418ef96ccfd848649a2e08d7f353ccd4%7C3406368982d44e89a3281ab79cc58d9d%7C0%7C0%7C637245413567073412&amp;sdata=9RNq%2FQC6FeFw5LE4%2BhHsTl2uGKys0Y5DstNEQS9HyA0%3D&amp;reserved=0

Sent from phone (please forgive typos)

From: Yurochko, Andrew D <AYuroc@lsuhsc.edu>
To: Orr, Anthony W. <aorr@lsuhsc.edu>
Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Kevil, Christopher <CKevil@lsuhsc.edu>
Woolard, Matthew D. <mwoola@lsuhsc.edu>
Sapp, Martin J <MSapp1@lsuhsc.edu>
Date: 5/8/2020 1:13:16 PM
Subject: Re: The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice | Nature

---

Thanks, yeah I had seen that. I was talking to one of my colleagues at UNC the other day about SARS. I know Ralph, we were actually just at study section together. It is certainly something to consider.
-Andrew

On 5/8/20, 8:05 AM, "Orr, Anthony W." <aorr@lsuhsc.edu> wrote:

See the attached preprint that Jeremy Kamil sent me this morning describing a mouse-adapted SARS-CoV-2 produced in the lab of Dr. Ralph Baric at UNC - Chapel Hill. This model would be far superior to the hACE2 transgenic mouse model, since the ACE2 receptor will be expressed in the right cells and at physiologically relevant levels. While this paper does not go beyond 7 days post-infection (probably to ensure rapid publication), it may (or may not) provide insight into the cardiovascular complications of COVID-19 infection. So my questions are:

1. Is this something we want to pursue?
2. Does anyone know Ralph Baric to potentially reach out for the virus?

Wayne

A. Wayne Orr, Ph.D.
Director, Center for Cardiovascular Diseases and Sciences
Professor and Director, Division of Research
Department of Pathology and Translational Pathobiology
Office: 318.675.5462
Lab: 318.675.5463

-----Original Message-----
From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Thursday, May 07, 2020 3:25 PM
To: Alexander, Jonathan Steven <JAlexa@lsuhsc.edu>; Kevil, Christopher <CKevil@lsuhsc.edu>; Orr, Anthony W. <aorr@lsuhsc.edu>
Subject: The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice | Nature

https://nam01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.nature.com%2Farticles%2Fs41586-020-2312-y&amp;data=02%7C01%7Cjkamil%40lsuhsc.edu%7C32f31b24c21041ab5a0b08d7f3519240%7C3406368982d44e89a3281ab79cc58d9d%7C0%7C0%7C637245403976154517&amp;sdata=%2F76TmrWFUpsw7VRSWXMNmyLBsX3U%2Bs7capdLVaju6hA%3D&amp;reserved=0

Sent from phone (please forgive typos)

From: Orr, Anthony W. <aorr@lsuhsc.edu>
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Kevil, Christopher <CKevil@lsuhsc.edu>
Woolard, Matthew D. <mwoola@lsuhsc.edu>
Yurochko, Andrew D <AYuroc@lsuhsc.edu>
Sapp, Martin J <MSapp1@lsuhsc.edu>
Date: 5/8/2020 1:05:25 PM
Subject: RE: The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice | Nature
Attachments: 2020.05.06.081497v1.full.pdf

See the attached preprint that Jeremy Kamil sent me this morning describing a mouse-adapted SARS-CoV-2 produced in the lab of Dr. Ralph Baric at UNC - Chapel Hill. This model would be far superior to the hACE2 transgenic mouse model, since the ACE2 receptor will be expressed in the right cells and at physiologically relevant levels. While this paper does not go beyond 7 days post-infection (probably to ensure rapid publication), it may (or may not) provide insight into the cardiovascular complications of COVID-19 infection. So my questions are:

1. Is this something we want to pursue?
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Wayne

A. Wayne Orr, Ph.D.
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-----Original Message-----
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Sent: Thursday, May 07, 2020 3:25 PM
To: Alexander, Jonathan Steven <JAlexa@lsuhsc.edu>; Kevil, Christopher <CKevil@lsuhsc.edu>; Orr, Anthony W. <aorr@lsuhsc.edu>
Subject: The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice | Nature

https://nam01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.nature.com%2Farticles%2Fs41586-020-2312-y&amp;data=02%7C01%7Cjkamil%40lsuhsc.edu%7C013d4c4914db42ec945b08d7f35079a0%7C3406368982d44e89a3281ab79cc58d9d%7C0%7C0%7C637245399316158432&amp;sdata=455Dvzqo5thp%2Bz3GokFNUCawp1WDMa6Hp87E78MvsHI%3D&amp;reserved=0

Sent from phone (please forgive typos)

From: Heguy, Adriana <Adriana.Heguy@nyulangone.org>
To: Cooper, Vaughn S. <vaughn.cooper@pitt.edu>
Date: 5/5/2020 6:25:50 PM
Subject: Re: GISAID's preferred Refseq is EPI_ISL_402124 (not Wuhan Hu-1)

This would be perfect!

Probably better if we run the scripts, Christian in my lab can do it. It's harder for me to transfer fastqs right now.

Sent from my iPhone, please excuse any typos

> On May 4, 2020, at 9:18 PM, Cooper, Vaughn S. <vaughn.cooper@pitt.edu> wrote:

> **[EXTERNAL]**
>
> Hi Adriana and Jeremy,
>
> Thanks for the intro and great response.
>
> (Switching to my Pitt account and my academic research hat. We're super proud of MiGS but it's primarily a sequencing biz.)
>
> I've been studying pathogen evolution and thinking about genetic diversity within hosts since my undergrad research >25 years ago (eventually published here) — when I was a budding virologist. I then worked on VSV with John Holland and then left and became a bacteriologist w/ Rich Lenski, and afterwards it's been mostly bacterial evolution of AMR and during chronic infections, then a recent foray into cancer evolution like you Adriana, — and then SARS-CoV-2 happened.
>
> I would be THRILLED to work with you to look for systematic, parallel patterns of within-patient diversity in your samples as well as ours (Pitt, LSU). There are at least two approaches:
>
> 1) I send you scripts / software and you have someone mine your FASTQ's for this information
> or
> 2) You send us FASTQ's and we do the same.
>
> If either work, I would like to propose a rapid response R01 supplement to do this.
>
> What do you think? Can we make this happen? We will need 1000's of genomes to do this.
>
> Best
> Vaughn
> ---
> Vaughn Cooper, Ph.D. Professor
> Microbiology and Molecular Genetics | Computational and Systems Biology | Biological Sciences
> Director, Center for Evolutionary Biology and Medicine
> University of Pittsburgh, School of Medicine
> 425 Bridgeside Point II, 450 Technology Drive Pittsburgh, PA 15219
> m: 603.988.7590 o: 412-624-1265

> lab | outreach | microbial genomics facility
>
>> On May 4, 2020, at 6:20 PM, Heguy, Adriana <Adriana.Heguy@nyulangone.org> wrote:
>>
>> Hi Jeremy, and hi Vaughn! Thanks for the intro, Jeremy. I'm actually much more excited by the mutations and the mixed variants than Matt, and I would be happy to work with Vaughn! I suspect Vaughn would need fast files and not the already deposited GISAID samples, but I'll let Vaughn say what he needs, and I'm sure we can find a way to collaborate!
>>
>> Funnily enough, Ralf Baric was a post-doc when I was a grad student at USC (back then, actual saber tooth tigers still roamed the LA basin, when I was a wide-eyed graduate student just landed from Uruguay...Small world. I remember Ralf very fondly, nice guy! If you talk to Ralf, tell him I said hi, and Filippo says hi too! Hopefully he'll remember me (and my then boyfriend, now husband for longer than I care to share, ha!.
>>
>> Best,
>>
>> Adriana
>>
>> ---
>>
>> **From:** Kamil, Jeremy P. <jkamil@lsuhsc.edu>
>> **Sent:** Monday, May 4, 2020 5:43PM
>> **To:** Heguy, Adriana
>> **Cc:** Vaughn Cooper
>> **Subject:** Re: GISAID's preferred Refseq is EPI_ISL_402124 (not Wuhan Hu-1)
>>
>> [EXTERNAL]
>> Hi Adriana,
>>
>> Introducing you to Vaughn Cooper, who has a lot of experience studying evolution of microbial genomes.   Vaughn is curious whether there might be some meaning underneath certain of the “mixed variants” that show up in Illumina data from SARS-CoV-2 clinical samples.. Vaughn completely gets that most variants are noise / not of great meaning. I’m sure that we are all also well aware that RNA viruses are sloppy copiers / generate quasispecies – but also that CoV’s encode an RNA replicase proofreading function/ nsp14, which allows for their “larger than the average RNA virus”-sized genome. But if you watch his youtube video (10 min), he uses the classic WWII fighter plane Survivorship Bias analogy to argue that regions of the virus that are underrepresented for SNPs might provide clues to new drug / therapeutic targets. https://www.youtube.com/watch?v=7gCY9tP981I
>>
>> IN ANY CASE: If you and Vaughn hit it off and end up being able to work together to answer some questions – or just to extract more public health / basic science value out of the huge amounts of NGS data you have sitting around, that’d be great.   No obligation to involve me!
>>
>> For my part, I do find it intriguing that certain viral genes might accumulate variants that approach 50% of the reads. I’d like to known if any of these show up often and are well represented already in extant viruses on GISAID.   Most these SNPs are no doubt noise / neutral / no effect on fitness.. but if by looking at lots of data sets you can begin to see ones that keep popping up over and over again, and are not explained away as artifacts of CoV RNA replication mechanisms, it would seem important to understand the biology.
>>
>> (i) Host encoded factors, like APOBEC, mutate HIV RNA by cytidine deamination. Are host

innate factors that do something similar against SARS2? Can you infer anything in high % read SNPs about host factors that might target the viral genome?

(ii) Certain SARS2 mutations might facilitate intrahost spread.. but are deleterious for horizontal transmission, so are there certain SNPs keep popping up in NP swabs as a decent sized minority of reads, but which are not maintained long term –i.e. underrepresented in consensus assemblies on GISAID

Although I'm just an "a la carte" human cytomegalovirus (herpes) virologist, who tends to chase sparkly things like a crow would.. I do have excellent friends in the Coronavirus community (e.g. Tony Fehr, at KU and my friend / collaborator Nat Moorman is in Ralph Baric's dept and collaborates extensively with Ralph).. so if you guys end up generating ideas from your combined data that need to be tested on the bench, I can certainly help make further introductions.

Viruses are super fascinating!

Cheers,

Jeremy

From: Vaughn Cooper <vaughn@migscenter.com>
Date: Monday, May 4, 2020 at 4:01 PM
To: Jeremy Kamil <jkamil@lsuhsc.edu>
Subject: Re: GISAID's preferred Refseq is EPI_ISL_402124 (not Wuhan Hu-1)

Following up — yes, I'll take your offer to make the connection with Heguy re: evolution within infections. I'm interested!

VC

> On May 3, 2020, at 3:32 PM, Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:
>
> So, Adriana Heguy at NYU Languone— she has about 500 genomes done already— told me that they too have evidence of "mixed variants" during infections... Since you and she both run big sequencing centers / projects, maybe I can introduce you to each other? (In the event y'all aren't already in touch).
>
> (I got to know her because she shared her hyb capture protocols with me way back in mid March, when I was first looking into how to get get this done.)
>
> Anyway, Vaughn, I'd love to see someone with your firepower / experience lead a study looking at this stuff. Also— guessing you've seen GISAID report from today that Steven got us both added to.. (from Cheryl Bennett, with Peter Bogner on the cc line!). If I understand the GISAID email correctly, there might actually be a minor number of mutants, say in Spike, that may affect transmission (F456L). (Caution, I know, is warranted). So I interesting to think of you and Adriana pooling primary / raw data from a ton of samples, to ask whatever questions you might be most interested in.

> > Apologies if you’re already 15 steps ahead of my thoughts here. :-).
> >
> > Finally, I am also friendly with Benhur Lee, who could directly assay using VSV pseudovirions if any variants affect entry. (He may already be doing this!).
> >
> > I’m also in contact within some folks in George Church’s lab.. they have a team of people developing LAMP-Seq, basically using RT-LAMP with incorporation of NGS for bar codes to enable massively parallel diagnostics using Illumina platforms. They will be getting sequence data when they make a call on a patient result (+ vs - for SARS) in a pooled run. So if there are regions of special interest for clades, or even possible Spike polymorphisms, they might benefit from hearing from you or lol.. simply looking at GISAID...
> >
> > surprisingly few people are integrating the molecular epidemiology and basic virology + diagnostics etc!
> >
> > Please let me know if you’d like me to put you in touch with anyone....
> >
> > Best,
> >
> > Jeremy
> >
> > Sent from phone (please forgive typos)
> >
> > > On May 1, 2020, at 9:45 PM, Vaughn Cooper <vaughn@migscenter.com> wrote:
> > >
> > > *EXTERNAL EMAIL: EVALUATE*
> > > Wow, Jeremy, you are hustling! Thanks for all of this backstory. Very valuable.
> > >
> > > Do you need us to reassemble against the bona fide reference?
> > > We noted the BaseSpace tool but were unsure if it passed muster. If they prefer, happy to use it.
> > >
> > > Finally, thanks for the continued support on the twitters. Dan and I owe you some swag!
> > >
> > > Cheers
> > > Vaughn
> > >
> > > > On May 1, 2020, at 9:20 PM, Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:
> > > >
> > > > Learned some useful stuff from a GISAID representative named Steven Meyers (a German based in Santa Monica.. Lucky guy),

who phoned me shortly after I submitted our data.. (mostly to correct a field I inadvertently left empty).   Learned lots of things from him.. He gave me the whole backstory of GIAID – Nancy Cox (CDC) and Peter Bogner (his boss) built GISAID.. for Flu.. and then along comes this..and China asked for their help in Dec or Jan or whatever..

Important point to share now.. GISAID actually aligns / recommends assemblies be done against EPI_ISL_402124. This is the authentic, traceable to a person and sample, first index case of the pneumonia in China. (Apparently NCBI is Crap for SARS-CoV-2.. Wuhan Hu-1 not traceable to a lab or person.. maybe I'm explaining it wrong but they don't like that file. Haha!

Other tidbits.:
VAUGHN..
(1) They were SUPER impressed by your coverage. Steven asked if some were typos (extra digit?).. they were not.
(2) if you use BaseSpace there's now a tool to directly assemble a SARS-CoV-2 genome out of the raw data (fastq.gz files)..
(3) he also told me a lot about the evolution from Horseshoe bats, which came from bats who roost in caves in jungle near Vietnam (if I remember what he said, correctly).
(4) Learned many wonderful things about GISAID..

they lock people out who don't acknowledge / cite the sequences they publish with! They are all about people getting credit for what they do.

they are going to make "EpiSet" files to allow others to download the same exact set of sequences that a person / publication uses in an analysis..

they are about to update their software to allow us to upload the entire raw data (reads) that were used to build a FASTA file !

Best,

Jeremy

Jeremy P. Kamil, Ph.D.
Associate Professor
Dept. of Microbiology and Immunology
LSU Health Sciences Center, Shreveport
Shreveport, Louisiana, USA
Ph: +1.318.759.7544
--
<EPI_ISL_402124.pdf><EPI_ISL_402124.fasta>

> > --------------
> > Vaughn Cooper, Ph.D.
> > Chief Scientific Officer
> > vaughn@migscenter.com

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Lee, Benhur <benhur.lee@mssm.edu>
Date: 1/28/2021 11:34:10 AM
Subject: Re: Curr Opinions in Virology Review

Benhur,

Certainly happy to. Skype or zoom would be great.
Authorship order however you like!

And f**k!! re Whelan's paper. :-(

Best,

Jeremy

Sent on the go. (Please forgive typos)

From: Lee, Benhur <benhur.lee@mssm.edu>
Sent: Thursday, January 28, 2021 2:15:33 AM
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Subject: Curr Opinions in Virology Review

Hi Jeremy,

This is a big ask, but I got the editor to agree to a change in topic.

Richard Plemper is in charge of a special issue in Current Opinions in Virology dedicated to SARS-CoV-2. I had agreed back in Nov to write a review a short one (2,000 words) on the controversy surrounding convalescent plasma.

But since we have spent so much time on collating and trying to understand these variants, I asked Richard if I could change the topic and write something on these variants. He agreed.

I was wondering if you would write it with me. A natural break-down would be genetics and function. You seem to have to have kept track of the evolution and lineage diversification ever since last Fall when we started making these point mutants. I only recently got into it from the structure-function side.

We would both be corresponding authors. You can go first or last, I don't care. The only triock is that the review is due in a week, but it's only 2,o00 words, I already made up the structural figures . . . you can explain the genetics of these strains and perhaps clarify the nomenclature. This is an *OPINION* piece rather than a straightforward review, so they want us to take a stand.

I figured it would be good place to ask for more national genomics sequencing as well as to opine on how to characterize these mutants properly.

I hope you're interested, and we can Skype briefly tomorrow? (*Curr Op in Virol* is a who's who in Virology, so it'll be a good venue for our work).

Best Regards,

Benhur

Benhur Lee, M.D.
Professor of Microbiology
Ward-Coleman Chair in Microbiology
Icahn School of Medicine at Mount Sinai
One Gustave L Levy Place #1124
New York, NY 10029
Office: (212) 241-2552 | Lab: (212) 241-9960

Lab Webpage: LeeLabVirus.Host **| Twitter** @VirusWhisperer

> On Jan 27, 2021, at 7:08 PM, Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:
>
> Great news re the shipment and re: the better titer!   We are gonna get there soon!
>
> Also.. not to distract anyone but I'll gonna drop something in the PS.. we may have a Louisiana "homegrown" variant here.. If any one of you knows how to model a Q677P into the spike, I'd love to know your thoughts as to potential effects on proteolytic processing of the PRRAR motif at 681-685.
>
> Jeremy
>
> PS:
>
> Keep this to yourselves, but potentially interesting lineage /variant.. showing up in sequences from all sorts of different collection sites (community testing, jail, nursing homes).. all appear to be B.1.2 GH / Nextstrain 20G.. we saw this mutation in our early Jan upload (viruses from up to Dec 18) but I didn't look closely how much there is.. now I realize it is a lot..   it *may* be out-competing others. It's about 15% of all our viruses in the latest batch.   Currently there are only 324 of these Q677P mutants on GISAID (from all over the world), and 166 many of them were collected / sequenced by EVTL lab.. 167 total from Louisiana (Air Force sequenced one, too). We are about to add another 56 from our latest sequencing (it is still being "accessioned"/ prepped for upload).   Interestingly, there is only one Q677P mutant (from 04 /2020, Spain) that shows up before November.   October and November starts to show a few in TX, CA, VA, NM, FL, then in Dec and Jan just blows up in Louisiana as well as in TX, MA, NM, + + + , Would be nice to have a professional gen-epi person run some analyses on this, but it's curious to observe as long as we don't over-interpret it..
>
> This Q677 is near the furin cleavage site (PRRAR 681-685). So it may be biologically interesting. Attaching a couple figures.. one is the phylogeny from our website.. but it does not include the new ones.
>
> Can any of you model it into the Spike to predict if the "kink" of the Pro might change proteolytic processing?   Image attached shows the rise of this mutation in our Louisiana build. .but this figure does not yet add our latest 56 new ones..
>
> 677-QTPTNSPRRAR-685
> to
> 677-PTPTNSPRRAR-685
> <image001.png>
>
> ---
>
> From: Ikegame, Satoshi <satoshi.ikegame@mssm.edu>
> Date: Wednesday, January 27, 2021 at 1:18 PM
> To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
> Cc: Mohammed Siddiquey <siddiquey27@gmail.com>, Lee, Benhur <benhur.lee@mssm.edu>, Brambilla, Luca (Student) <luca.brambilla@icahn.mssm.edu>, Ivanov, Stanimir S. <sivano@lsuhsc.edu>, Dragoi, Ana

Maria <adrag1@lsuhsc.edu>, Kasopefoluwa Oguntuyo <sope.oguntuyo@icahn.mssm.edu>, Stevens, Christian (Student) <christian.stevens@icahn.mssm.edu>
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

We will be shipping B.1.351-GFP-VSV-CoV2 and VOC wt-GFP (18 tubes each).
Tracking number is 816009348652.

Titer of B1.1.7-GFP was 8*10^4 IU/ml (I made a mistake in calculation.)

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Wednesday, January 27, 2021 8:07:26 AM
To: Ikegame, Satoshi
Cc: Mohammed Siddiquey; Lee, Benhur; Brambilla, Luca (Student); Ivanov, Stanimir S.; Dragoi, Ana Maria; Kasopefoluwa Oguntuyo; Stevens, Christian (Student)
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Hi Satoshi,

1. That’s fantastic if you can ship them today! Helps us move faster. I’ll look at the preprint ASAP.

2. By the way, we just got 367 new CoV2 sequences in from late Dec - Jan 19. Not on GISAID yet — I have to process them for upload but many are:
P681H — shared with B.1.1.7
or
Q677H — allegedly shows up in new US variants of concern
or
Q677P

Here’s an RTF file if any of you want to peruse it. I think we *may* have some new locally grown variants kicking around in the USA! https://www.dropbox.com/s/2bbzfgokbb8kwzd/muts_summ_20210127.rtf?dl=0

Summary of all mutations in our recent batch https://www.dropbox.com/s/l4hiyii3dwom6zr/myresults.csv?dl=0

Best,

Jeremy

Sent from phone (please forgive typos)

On Jan 27, 2021, at 6:50 AM, Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:

https://www.biorxiv.org/content/10.1101/2021.01.25.428137v2
New study is released from Columbia university.

Only 22 serum were tested, but quite similar to ours.

I believe we can ship VOCwt-GFP (just collected and put into liquid nitrogen for quick freezing) and B1.358-GFP today.

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Ikegame, Satoshi
Sent: Tuesday, January 26, 2021 6:05:25 PM
To: Kamil, Jeremy P.
Cc: Mohammed Siddiquey; Lee, Benhur; Brambilla, Luca (Student); Ivanov, Stanimir S.; Dragoi, Ana Maria
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Dear Jeremy.

For growth assay.

1; I recommend to start from MOI=0.01 using 12 or 6 well plate.

2; I haven't checked growth in Vero or Vero-TM2 cells, but 72 hours must be enough to reach peak titer.

Replace half of medium twice a day up to 72 hours (every 24 hours check may fail to find peak because of fast growth).

3; I believe that we do not have to evaluate the cell associated viruses by scrape /freeze and thaw.

I believe that titer in supernatant will be fine unless special reason.

(To evaluate cell associated virus, we need sonicator. But I could not find good one for my measles virus project.)

4; For comparison of growth, I do not recommend to use different colored virus.

Sensitivity or brightness of FP may result in underestimation of titer (especially in E2-crimson) and make interpretation difficult.

For neutralizing assay by RBD-Fc;

I recommend to start from x 1/40 or 1/80 dilution (final concentration in the medium; supposed to be 145 or 72.5 ug/ml).

Then serial dilution of x1/4 (up to x1/4^7 = x1/16384).

GFP wont' be visible at > 10ug/ml.

(Actually we keep RBD-Fc in 4 degrees, but remains effective for several month.)

Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Tuesday, January 26, 2021 4:57:03 PM
To: Ikegame, Satoshi
Cc: Mohammed Siddiquey; Lee, Benhur; Brambilla, Luca (Student); Ivanov, Stanimir S.; Dragoi, Ana Maria
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Hi Satoshi and friends.. a few things (two are questions).

1. We got the B.1.1.7 GFP virus shipment. Thanks!

2. For the replication kinetics curves, please let us know your favorite approach or if we should just emulate what Sean Whelan or Kartik's paper's did (I'm assuming they did this)..
    a. What MOI should we use to infect the VERO-CCL81?
    b. What time points to take? I think a 48-72 h curve should be plenty even at low MOI.. but less if we go with higher MOI.

        c. We would -80 deg C freeze thaw 1X scaped cells to get cell associated + cell free titer all in one and obtain read out using TCID50.
        d. Idea is to make a curve for each of the mutants and wild-types in different color backgrounds.

3. We have C-T's Batch 5 RBD-Fc (in PBS), Conc: 2.9 µg/µL in ~ 500 µL, Total amount: 1450 µg. It is still in the original tube.
    a. If you have an ideal dilution series we should use on our assay plates as "internal standard" please let us know.
    b. My plan is to ask Siddiquey to make 20 aliquots of ~25 uL each. Or should I ask him to dilute it to ~1.5 mL of 1 ug/uL (1 mg/mL) in 25 mM HEPES Buffered Saline pH 7.5 + 10% glycerol + 0.05 mg/mL BSA. This way he could freeze back *snap freeze on liquid nitrogen—and make ~30 aliquots with each being ~50 uL.

Cheers,

Jeremy

From: Ikegame, Satoshi <satoshi.ikegame@mssm.edu>
Date: Tuesday, January 26, 2021 at 8:18 AM
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Cc: Mohammed Siddiquey <siddiquey27@gmail.com>, Lee, Benhur <benhur.lee@mssm.edu>, Brambilla, Luca (Student) <luca.brambilla@icahn.mssm.edu>, Ivanov, Stanimir S. <sivano@lsuhsc.edu>, Dragoi, Ana Maria <adrag1@lsuhsc.edu>
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Dear Jeremy.

I just tittered B.1.1.7-GFP virus.

Titer was only 8x 10^3 IU/ml (Sample collection timing might have been a little bit earlier).

However, stock can make > 500 GFP events/well in 96 well at x1/10 dilution.

Sequence of genome RNA seems fine at least in spike-ORF.

B1.351-GFP has been collected this morning, and VOCwt-GFP will be collected tomorrow.

I believe we will ship both on next Monday.

Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Monday, January 25, 2021 7:03:13 PM
To: Ikegame, Satoshi
Cc: Mohammed Siddiquey; Lee, Benhur; Haas, Griffin (Student); Brambilla, Luca (Student); Ivanov, Stanimir S.; Dragoi, Ana Maria
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

This sounds really great, Satoshi. :-)

Sent from phone (please forgive typos)

> On Jan 25, 2021, at 5:31 PM, Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:
>
> I think mScarlet1 wild type is Florian's codon optimized sequence.
>
> So I will try to rescue VOC wild type-mScarlet1.
>
> (If we finish assay within 12hours (single round infection), codon selection won't be so problematic.)
>
> Satoshi Ikegame
>
> mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Monday, January 25, 2021 3:10:39 PM
To: Ikegame, Satoshi
Cc: Mohammed Siddiquey; Lee, Benhur; Haas, Griffin (Student); Brambilla, Luca (Student); Ivanov, Stanimir S.; Dragoi, Ana Maria
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

This is great news, Satoshi. I do think Stan likes to use mScarlet-I for wild type.

Siddiquey: please make sure we have plenty of mScarlet WT D614G Del21 grown up!

Best,

Jeremy

Sent from phone (please forgive typos)

> On Jan 25, 2021, at 2:03 PM, Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:
>
> We are about to ship B.1.1.7-GFP-VSV-CoV2 (I did not count

number of tubes, but > 15 tubes).

Tracking number is 816009348663.

VOC wild type-GFP and B1.358-GFP are now being amplified, they will be ready by the end of week.

We will also work on mScarlet-1 and E2-crimson later.

But I slightly remember that Stan is planning to change mScarlet1 with other FP.

Let me know if you are planning to use different colors other than mScarlet1.

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Sunday, January 24, 2021 9:49:14 PM
To: Ikegame, Satoshi
Cc: Mohammed Siddiquey; Lee, Benhur; Haas, Griffin (Student); Brambilla, Luca (Student)
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Look forward to reading this. Reminds me of findings from the paper from Vineet Menachery (Benhur is a coauthor too)..

I wonder if there isn't also an enhancement of cell free infectivity when polybasic site is mutated?!

IMO worth looking at, at some point...

Sent from phone (please forgive typos)

> On Jan 24, 2021, at 6:17 PM, Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:
>
> https://www.biorxiv.org/content/10.1101/2021.01.22.427802v1
>
> This paper may be interesting for you.
> Cleavage site mutations happen without protease expression, and increase entry into VeroE6 cells.
>
> Satoshi Ikegame
>
> mail to:
>
> satoshi.ikegame@mssm.edu
>
> Department of Microbiology, Icahn School of Medicine at Mount Sinai.
>
> ---
>
> **From:** Kamil, Jeremy P. <jkamil@lsuhsc.edu>

Sent: Sunday, January 24, 2021 6:29:30 PM
To: Ikegame, Satoshi; Mohammed Siddiquey
Cc: Lee, Benhur; Haas, Griffin (Student); Brambilla, Luca (Student)
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Hi Satoshi,

Thanks for your update, too. .that is super exciting. Based on our observation that the only mutation we saw in Sean Whelan’s virus was the PRRAR à PRRAS mutation, I doubt you would detect any mutations arising quickly in the freshly recovered VSV-B.1.1.7 Spike but never hurts to have the data (in case reviewers ask for it.)

In any case, we are excited to test it here for escape from neutralization!

Best,

Jeremy

From: Satoshi Ikegame <satoshi.ikegame@mssm.edu>
Date: Sunday, January 24, 2021 at 5:26 PM
To: Jeremy Kamil <jkamil@lsuhsc.edu>, Mohammed Siddiquey <siddiquey27@gmail.com>
Cc: Benhur Lee <benhur.lee@mssm.edu>, Griffin Haas <griffin.haas@icahn.mssm.edu>, "Brambilla, Luca (Student)" <luca.brambilla@icahn.mssm.edu>
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Dear Jeremy.

Thank you for your update.

I just collected B.1.1.7 VSV-CoV2GFP.

I will ship tomorrow or on Tuesday approx 20 tubes.

Luca will work on sequencing genome RNA to see if no mutation happens in spike gene.

This will be done in several days, and will let you know result.

Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Sunday, January 24, 2021 1:54:25 PM
To: Mohammed Siddiquey; Ikegame, Satoshi
Cc: Lee, Benhur; Haas, Griffin (Student)
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Hi Satoshi, (**Siddiquey**: please add this info to the glycerol stocks in the Google Drive).

Based on the latest data that came in from genewiz re-runs, we can now say these two also look good:

- pEMC-VSV-mScarlet full 501Y.V2 / B.1.351 # clone 1
- pEMC-VSV-eGFP_501Y.V2 (S African) RBD # clone 2

To provide a re-cap / summary including all the clones that Siddiquey shipped which appear to be good..

1. pEMC-VSV-eGFP_501Y.V2 (S African) RBD # clone 1
2. pEMC-VSV-eGFP_501Y.V2 (S African) RBD # clone 2

Note the above two are RBD only "trifecta" of K417N/E484K /N501Y.. and will not need to use VOC_CTRL as a control.

3. pEMC-VSV-eGFP FULL 501Y.V2 (S African) # clone 2. This is FULL length clone.. will need VOC_CTRL for best data.
    a. ** I can't read the very end of the gBlock because the Rev primer failed. but it is probably fine!
4. pEMC-VSV-mScarlet full 501Y.V2 / B.1.351 # clone 1
5. pEMC-VSV-eGFP VOC_CTRL # clone 2
    a. note, SILENT wobble mutation of 3rd position of Ser477 (TCT to TCC).. but no expected effect…
6. pEMC-VSV-mScarlet VOC_CTRL # clone 3, looks perfect!

Please let me know if you'd like me to email you the sequence files or alignments.

Cheers,

Jeremy

From: Jeremy Kamil <jkamil@lsuhsc.edu>
Date: Friday, January 22, 2021 at 1:47 PM
To: Mohammed Siddiquey <siddiquey27@gmail.com>, Satoshi Ikegame <satoshi.ikegame@mssm.edu>
Cc: Benhur Lee <benhur.lee@mssm.edu>, Griffin Haas <griffin.haas@icahn.mssm.edu>
Subject: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Hi Satoshi,

Of the plasmids and bacteria that Siddiquey shipped on Tuesday, here's our results from Genewiz..

These clones look **GOOD or LIKELY GOOD** by Sanger Sequencing.

1. pEMC-VSV-eGFP_501Y.V2 (S African) RBD # clone 1 **#RBD only!** This one will not need to use VOC_CTRL as a control.
    2. pEMC-VSV-eGFP FULL 501Y.V2 (S African) # clone 2. This is FULL length clone.. will need VOC_CTRL for best data.
        a. ** I can't read the very end of the gBlock because the Rev primer failed. but it is probably fine!
    3. pEMC-VSV-eGFP VOC_CTRL # clone 2
        a. note, SILENT wobble mutation of 3rd position of Ser477 (TCT to TCC).. both codons pretty much equally abundant.. no expected effect!
    4. pEMC-VSV-mScarlet VOC_CTRL # clone 3, looks perfect!

These clones are **BAD:**

5. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 2.
    a. Missense mutation of V610I (numbering per Wuhan Hu-1 REF Spike) …this would be Val606Ile in B.1.351 (501Y.V2) Spike
    b. Silent unintended GGC to GGT wobble mutation at Gly codon 252 (Wuhan REF numbering).. this would be Gly249 of actual 501Y2 translation product.

These clones I can't tell yet..

1. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 1
    a. can't read ATG at 5'-end due to smear.
2. Some others didn't sequence well yet either..

Here is the raw data https://www.dropbox.com/s/2ebdfnpm42rkfgv/JEREMYKAMIL01-22-2021_182552_347.zip?dl=0

And the alignments: https://www.dropbox.com/sh/27uz4816j3xolll/AACc5_7RMFJ8jP_nT4aFAgfsa?dl=0 (some are macvector format but also FASTA are provided).

Cheers,

Jeremy

From: Mohammed Siddiquey <siddiquey27@gmail.com>
Date: Tuesday, January 19, 2021 at 7:06 PM
To: Ikegame, Satoshi

<satoshi.ikegame@mssm.edu>
Cc: Lee, Benhur <benhur.lee@mssm.edu>, Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Subject: Re: SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor | bioRxiv

EXTERNAL EMAIL: EVALUATE

Hi Satoshi,

I am sending you the following today. The tracking number is 772672573621.

Please let me know after receiving.

Thank you.

Siddiquey

DNA (20 ul, >100 ng /ul):

1. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 1
2. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 2
3. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 3

4. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 1

5. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 2

6. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 3

7. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 1

8. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 2

9. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 3

10. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 1

11. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 2

12. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 3

13. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 1

14. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 2

15. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 3

16. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 1

17. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 2

18. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 3

19. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 1

20. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 2

21. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 3

Bacteria (50 ul):

22. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 1

23. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 2

24. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 3

25. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 1

26. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 2

27. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 3

28. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 1

29. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 2

30. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 3

31. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 1

32. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 2

33. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 3

34. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 1

35. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 2

36. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 3

37. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 1

38. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 2

39. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 3

40. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 1

41. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 2

42. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 3

On Thu, Jan 14, 2021 at 1:49 PM Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:

> I noticed I cannot start transformation until you get sequence result.
>
> Sorry for the confusion.
>
> Satoshi Ikegame
>
> mail to:
>
> satoshi.ikegame@mssm.edu
>
> Department of Microbiology, Icahn School of Medicine at Mount Sinai.
>
> ---
>
> **From:** Ikegame, Satoshi
> **Sent:** Thursday, January 14, 2021 2:46:44 PM
> **To:** Mohammed Siddiquey; Lee, Benhur
> **Cc:** Kamil, Jeremy P.
> **Subject:** Re: SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor | bioRxiv
>
> Hi Shiddiquey.

I just received package and will start transformation.

Thank you.

Next time, could you send us bacteria (20ul LB medium in tube) in addition to plasmid if possible?

Best,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Mohammed Siddiquey <siddiquey27@gmail.com>
Sent: Wednesday, January 13, 2021 8:46:14 PM
To: Lee, Benhur
Cc: Ikegame, Satoshi; Kamil, Jeremy P.
Subject: Re: SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor | bioRxiv

Hi Satoshi,

I have sent you the following gBlocks and DNA today. Tracking number is 772622125267.

Please let me know after receiving. I am retransforming 417_501 NKY mutant. Colonies from my first transformation are a little weird. Hopefully I can send you some good clones soon.

Thank you.

Siddiquey

gBlocks:

1. Bsu Sph 417_501 NKY (gBlocks, 10 ng/ul), 20 ul
2. B.1.1.7   (gBlock 1, 10 ng/ul) 20 ul
3. B.1.1.7   (gBlock 2, 10 ng/ul) 20 ul

DNA (20 ul, 100 ng /ul):

4. pEMC-VSV-eGFP D614G_B.1.1.7 # clone 1
5. pEMC-VSV-eGFP D614G_B.1.1.7 # clone 2
6. pEMC-VSV-eGFP D614G_B.1.1.7 # clone 3
7. pEMC-VSV-eGFP D614G_B.1.1.7 # clone 4
8. pEMC-VSV-mScarlet D614G_B.1.1.7 # clone 1
9. pEMC-VSV-mScarlet D614G_B.1.1.7 # clone 2
10. pEMC-VSV-mScarlet D614G_B.1.1.7 # clone 3
11. pEMC-VSV-mScarlet D614G_B.1.1.7 # clone 4
12. pEMC-VSV-E2_Crimson D614G_B.1.1.7 # clone 1

13. pEMC-VSV-E2_Crimson D614G_B.1.1.7 # clone 2

On Mon, Jan 11, 2021 at 5:33 PM Lee, Benhur <benhur.lee@mssm.edu> wrote:

> Hi Jeremy,
>
> I got two random cold calls (emails) today asking me if I have the 501.V2 variant: N501Y, K417N, E484K? One was from a colleague with VA connections saying they want to test 55 COVID-19 positive sera.
>
> What the hell are people thinking? That you (we) make & rescue them just for fun so others can test them? (How do they know we're even doing this?)
>
> My answer is going to be "No, but my collaborators *may* have made them and they are testing them on their panel of sera".
>
> Sounds good? We better get this out soon. Meanwhile, I just asked Satoshi to start virus evolution expt using sera on our rcVSV-CoV2-S. Since we already have the individual N501Y, delta-F140, and E484K *individual* mutants, do you think starting from one of these mutants will be faster?

(**Satoshi, attached is the paper I was talking to you about earlier . . . it took them 5-8 days for each passage and 7 passages (45 days) to get their first mutant and 14 passages (85-95 days) to get a completely resistant virus: delta F140, E484K, 248-KTRNKSTSRRE 11aa insertion. I think we can do each passage at 24-36 hrs and get resistant mutants in 2 weeks, especially since VSV mutates faster than SARS-CoV-2).**

Anyway, seems like everyone is rushing this, no more being number two after all the hard work we all have put into making this system work. BTW, I find it impossible that a 11-aa insertion spontaneously occurs. Don' you think it must be present a very rare frequency or some sort of recombination event occurred?

Should we have a joint call to determine priorities once your study section is over?

Benhur

Benhur Lee, M.D.
Professor of Microbiology
Ward-Coleman Chair in Microbiology
Icahn School of Medicine at Mount Sinai
One Gustave L Levy Place #1124
New York, NY 10029
Office: (212) 241-2552 | Lab: (212) 241-9960

Lab Webpage: LeeLabVirus.Host **| Twitter** @VirusWhisperer

> On Jan 11, 2021, at 2:56 PM, Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:
>
> N501Y, N501Y+E484K have higher affinity.
>
> This cannot be found in my VSV-CoV2spike.
>
> We may need to condider different kind of assay to evaluate S vs ACE2 affinity.
>
> It is good to know that you've already cloned 501V1 and 501V2.

Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Monday, January 11, 2021 2:44:40 PM
To: Lee, Benhur; Haas, Griffin (Student); Ikegame, Satoshi; Mohammed Siddiquey; Hung, Chuan-tien; Dragoi, Ana Maria
Subject: SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor | bioRxiv

USE CAUTION: External Message.

https://www.biorxiv.org/content/10.1101/2021.01.06.425392v2

https://twitter.com/_b_meyer/status/1348695525483290625?s=21

“ While the single 501Y mutations causes a ~2.5x higher affinity to Ace2, the double mutation with E484K results in a ~13x higher affinity to Ace2 compared to the WT version.”

<image0.jpeg>

We have this cloned already (as RBD) of S Africa variant B.1.351, in the GFP VSV Spike backbone.

Cheers,

Jeremy

Sent from phone (please forgive typos)

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Ikegame, Satoshi <satoshi.ikegame@mssm.edu>
Date: 1/28/2021 12:08:21 AM
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Great news re the shipment and re: the better titer! We are gonna get there soon!

Also.. not to distract anyone but I'll gonna drop something in the PS.. we may have a Louisiana "homegrown" variant here.. If any one of you knows how to model a Q677P into the spike, I'd love to know your thoughts as to potential effects on proteolytic processing of the PRRAR motif at 681-685.

Jeremy

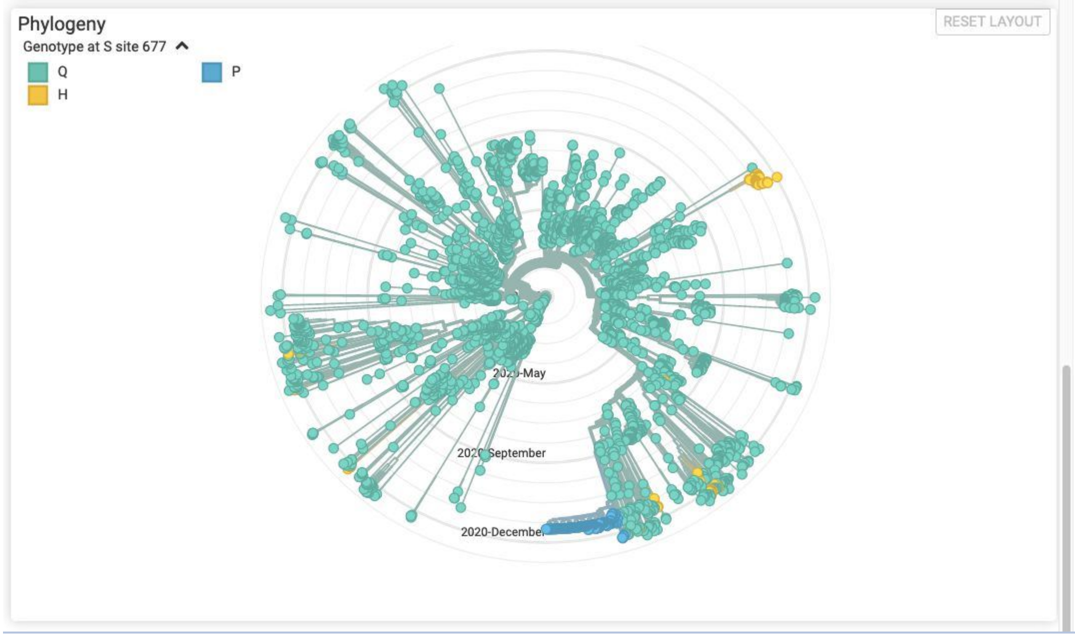
PS:

Keep this to yourselves, but potentially interesting lineage /variant.. showing up in sequences from all sorts of different collection sites (community testing, jail, nursing homes).. all appear to be B1.2 GH / Nextstrain 20G.. we saw this mutation in our early Jan upload (viruses from up to Dec 18) but I didn't look closely how much there is.. now I realize it is a lot.. it *may* be out-competing others. It's about 15% of all our viruses in the latest batch. Currently there are only 324 of these Q677P mutants on GISAID (from all over the world), and 166 many of them were collected / sequenced by EVTL lab.. 167 total from Louisiana (Air Force sequenced one, too). We are about to add another 56 from our latest sequencing (it is still being "accessioned"/ prepped for upload). Interestingly, there is only one Q677P mutant (from 04/2020, Spain) that shows up before November. October and November starts to show a few in TX, CA, VA, NM, FL, then in Dec and Jan just blows up in Louisiana as well as in TX, MA, NM, + + + , Would be nice to have a professional gen-epi person run some analyses on this, but it's curious to observe as long as we don't over-interpret it..

This Q677 is near the furin cleavage site (PRRAR 681-685). So it may be biologically interesting. Attaching a couple figures.. one is the phylogeny from our website.. but it does not include the new ones.

Can any of you model it into the Spike to predict if the "kink" of the Pro might change proteolytic processing? Image attached shows the rise of this mutation in our Louisiana build. .but this figure does not yet add our latest 56 new ones..

677-QTPTNSPRRAR-685
to
677-PTPTNSPRRAR-685



From: Ikegame, Satoshi <satoshi.ikegame@mssm.edu>
Date: Wednesday, January 27, 2021 at 1:18 PM
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Cc: Mohammed Siddiquey <siddiquey27@gmail.com>, Lee, Benhur <benhur.lee@mssm.edu>, Brambilla, Luca (Student) <luca.brambilla@icahn.mssm.edu>, Ivanov, Stanimir S. <sivano@lsuhsc.edu>, Dragoi, Ana Maria <adrag1@lsuhsc.edu>, Kasopefoluwa Oguntuyo <sope.oguntuyo@icahn.mssm.edu>, Stevens, Christian (Student) <christian.stevens@icahn.mssm.edu>
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

We will be shipping B.1.351-GFP-VSV-CoV2 and VOC wt-GFP (18 tubes each).
Tracking number is 816009348652.

Titer of B1.1.7-GFP was 8*10^4 IU/ml (I made a mistake in calculation.)

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Wednesday, January 27, 2021 8:07:26 AM
To: Ikegame, Satoshi
Cc: Mohammed Siddiquey; Lee, Benhur; Brambilla, Luca (Student); Ivanov, Stanimir S.; Dragoi, Ana Maria; Kasopefoluwa Oguntuyo; Stevens, Christian (Student)
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Hi Satoshi,

1. That's fantastic if you can ship them today! Helps us move faster. I'll look at the preprint ASAP.

2. By the way, we just got 367 new CoV2 sequences in from late Dec - Jan 19. Not on GISAID yet — I have to process them for upload but many are:
P681H — shared with B.1.1.7
or
Q677H — allegedly shows up in new US variants of concern
or
Q677P

Here's an RTF file if any of you want to peruse it. I think we *may* have some new locally grown variants kicking around in the USA! https://www.dropbox.com/s/2bbzfgokbb8kwzd/muts_summ_20210127.rtf?dl=0

Summary of all mutations in our recent batch https://www.dropbox.com/s/l4hiyii3dwom6zr/myresults.csv?dl=0

Best,

Jeremy

Sent from phone (please forgive typos)

> On Jan 27, 2021, at 6:50 AM, Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:
>
> https://www.biorxiv.org/content/10.1101/2021.01.25.428137v2
> New study is released from Columbia university.
>
> Only 22 serum were tested, but quite similar to ours.
>
> I believe we can ship VOCwt-GFP (just collected and put into liquid nitrogen for quick freezing) and B1.358-GFP today.
>
> Satoshi Ikegame
>
> mail to:
>
> satoshi.ikegame@mssm.edu
>
> Department of Microbiology, Icahn School of Medicine at Mount Sinai.
>
> ---
>
> From: Ikegame, Satoshi
> Sent: Tuesday, January 26, 2021 6:05:25 PM
> To: Kamil, Jeremy P.
> Cc: Mohammed Siddiquey; Lee, Benhur; Brambilla, Luca (Student); Ivanov, Stanimir S.; Dragoi, Ana Maria
> Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Dear Jeremy.

For growth assay.

1; I recommend to start from MOI=0.01 using 12 or 6 well plate.

2; I haven't checked growth in Vero or Vero-TM2 cells, but 72 hours must be enough to reach peak titer.

Replace half of medium twice a day up to 72 hours (every 24 hours check may fail to find peak because of fast growth).

3; I believe that we do not have to evaluate the cell associated viruses by scrape/freeze and thaw.

I believe that titer in supernatant will be fine unless special reason.

(To evaluate cell associated virus, we need sonicator. But I could not find good one for my measles virus project.)

4; For comparison of growth, I do not recommend to use different colored virus.

Sensitivity or brightness of FP may result in underestimation of titer (especially in E2-crimson) and make interpretation difficult.

For neutralizing assay by RBD-Fc;

I recommend to start from x 1/40 or 1/80 dilution (final concentration in the medium; supposed to be 145 or 72.5 ug/ml).

Then serial dilution of x1/4 (up to x1/4^7 = x1/16384).

GFP wont' be visible at > 10ug/ml.

(Actually we keep RBD-Fc in 4 degrees, but remains effective for several month.)

Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Tuesday, January 26, 2021 4:57:03 PM
To: Ikegame, Satoshi
Cc: Mohammed Siddiquey; Lee, Benhur; Brambilla, Luca (Student); Ivanov, Stanimir S.; Dragoi, Ana Maria
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Hi Satoshi and friends.. a few things (two are questions).

1. We got the B.1.1.7 GFP virus shipment. Thanks!

2. For the replication kinetics curves, please let us know your favorite approach or if we should just emulate what Sean Whelan or Kartik's paper's did (I'm assuming they did this)..
    a. What MOI should we use to infect the VERO-CCL81?
    b. What time points to take? I think a 48-72 h curve should be plenty even at low MOI.. but less if we go with higher MOI.
    c. We would -80 deg C freeze thaw 1X scaped cells to get cell associated + cell free titer all in one and obtain read out using TCID50.
    d. Idea is to make a curve for each of the mutants and wild-types in different color backgrounds.

3. We have C-T's Batch 5 RBD-Fc (in PBS), Conc: 2.9 µg/µL in ~ 500 µL, Total amount: 1450 µg. It is still in the original tube.
    a. If you have an ideal dilution series we should use on our assay plates as "internal standard" please let us know.
    b. My plan is to ask Siddiquey to make 20 aliquots of ~25 uL each. Or should I ask him to dilute it to ~1.5 mL of 1 ug/uL (1 mg/mL) in 25 mM HEPES Buffered Saline pH 7.5 + 10% glycerol + 0.05 mg/mL BSA. This way he could freeze back *snap freeze on liquid nitrogen—and make ~30 aliquots with each being ~50 uL.

Cheers,

Jeremy

From: Ikegame, Satoshi <satoshi.ikegame@mssm.edu>
Date: Tuesday, January 26, 2021 at 8:18 AM
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Cc: Mohammed Siddiquey <siddiquey27@gmail.com>, Lee, Benhur <benhur.lee@mssm.edu>, Brambilla, Luca (Student) <luca.brambilla@icahn.mssm.edu>, Ivanov, Stanimir S. <sivano@lsuhsc.edu>, Dragoi, Ana Maria <adrag1@lsuhsc.edu>
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Dear Jeremy.

I just tittered B.1.1.7-GFP virus.

Titer was only 8x 10^3 IU/ml (Sample collection timing might have been a little bit earlier).

However, stock can make > 500 GFP events/well in 96 well at x1/10 dilution.

Sequence of genome RNA seems fine at least in spike-ORF.

B1.351-GFP has been collected this morning, and VOCwt-GFP will be collected tomorrow.

I believe we will ship both on next Monday.

Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Monday, January 25, 2021 7:03:13 PM
To: Ikegame, Satoshi
Cc: Mohammed Siddiquey; Lee, Benhur; Haas, Griffin (Student); Brambilla, Luca (Student); Ivanov, Stanimir S.; Dragoi, Ana Maria
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

This sounds really great, Satoshi. :-)

Sent from phone (please forgive typos)

On Jan 25, 2021, at 5:31 PM, Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:

I think mScarlet1 wild type is Florian's codon optimized sequence.

So I will try to rescue VOC wild type-mScarlet1.

(If we finish assay within 12hours (single round infection), codon selection won't be so problematic.)

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Monday, January 25, 2021 3:10:39 PM
To: Ikegame, Satoshi
Cc: Mohammed Siddiquey; Lee, Benhur; Haas, Griffin (Student); Brambilla, Luca (Student); Ivanov, Stanimir S.; Dragoi, Ana Maria
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

This is great news, Satoshi. I do think Stan likes to use mScarlet-I for wild type.

Siddiquey: please make sure we have plenty of mScarlet WT D614G Del21 grown up!

Best,

Jeremy

Sent from phone (please forgive typos)

> On Jan 25, 2021, at 2:03 PM, Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:
>
> We are about to ship B.1.1.7-GFP-VSV-CoV2 (I did not count number of tubes, but > 15 tubes).
>
> Tracking number is 816009348663.
>
> VOC wild type-GFP and B1.358-GFP are now being amplified, they will be ready by the end of week.
>
> We will also work on mScarlet-1 and E2-crimson later.
>
> But I slightly remember that Stan is planning to change mScarlet1 with other FP.
>
> Let me know if you are planning to use different colors other than mScarlet1.
>
> Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Sunday, January 24, 2021 9:49:14 PM
To: Ikegame, Satoshi
Cc: Mohammed Siddiquey; Lee, Benhur; Haas, Griffin (Student); Brambilla, Luca (Student)
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Look forward to reading this. Reminds me of findings from the paper from Vineet Menachery (Benhur is a coauthor too)..

I wonder if there isn't also an enhancement of cell free infectivity when polybasic site is mutated?!

IMO worth looking at, at some point...

Sent from phone (please forgive typos)

On Jan 24, 2021, at 6:17 PM, Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:

https://www.biorxiv.org/content/10.1101/2021.01.22.427802v1

This paper may be interesting for you.
Cleavage site mutations happen without protease expression, and increase entry into VeroE6 cells.

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Sunday, January 24, 2021 6:29:30 PM
To: Ikegame, Satoshi; Mohammed Siddiquey
Cc: Lee, Benhur; Haas, Griffin (Student); Brambilla, Luca (Student)
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Hi Satoshi,

Thanks for your update, too. .that is super exciting. Based on our observation that the only mutation we saw in Sean Whelan's virus was the PRRAR à PRRAS mutation, I doubt you would detect any mutations arising quickly in the freshly recovered VSV-B.1.1.7 Spike but never hurts to have the data (in case reviewers ask for it.)

In any case, we are excited to test it here for escape from neutralization!

Best,

Jeremy

From: Satoshi Ikegame <satoshi.ikegame@mssm.edu>
Date: Sunday, January 24, 2021 at 5:26 PM
To: Jeremy Kamil <jkamil@lsuhsc.edu>, Mohammed Siddiquey <siddiquey27@gmail.com>
Cc: Benhur Lee <benhur.lee@mssm.edu>, Griffin Haas <griffin.haas@icahn.mssm.edu>, "Brambilla, Luca (Student)" <luca.brambilla@icahn.mssm.edu>
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Dear Jeremy.

Thank you for your update.

I just collected B.1.1.7 VSV-CoV2GFP.

I will ship tomorrow or on Tuesday approx 20 tubes.

Luca will work on sequencing genome RNA to see if no mutation happens in spike gene.

This will be done in several days, and will let you know result.

Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Sunday, January 24, 2021 1:54:25 PM
To: Mohammed Siddiquey; Ikegame, Satoshi
Cc: Lee, Benhur; Haas, Griffin (Student)
Subject: Re: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Hi Satoshi, (**Siddiquey**: please add this info to the glycerol stocks in the Google Drive).

Based on the latest data that came in from genewiz re-runs, we can now say these two also look good:

- pEMC-VSV-mScarlet full 501Y.V2 / B.1.351 # clone 1
- pEMC-VSV-eGFP_501Y.V2 (S African) RBD # clone 2

To provide a re-cap / summary including all the clones that Siddiquey shipped which appear to be good..

1. pEMC-VSV-eGFP_501Y.V2 (S African) RBD # clone 1
2. pEMC-VSV-eGFP_501Y.V2 (S African) RBD # clone 2

Note the above two are RBD only "trifecta" of K417N/E484K/N501Y.. and will not need to use VOC_CTRL as a control.

3. pEMC-VSV-eGFP FULL 501Y.V2 (S African) # clone 2. This is FULL length clone.. will need VOC_CTRL for best data.
   a. ** I can't read the very end of the gBlock because the Rev primer failed. but it is probably fine!
4. pEMC-VSV-mScarlet full 501Y.V2 / B.1.351 # clone 1
5. pEMC-VSV-eGFP VOC_CTRL # clone 2
   a. note, SILENT wobble mutation of 3rd position of Ser477 (TCT to TCC).. but no expected effect…
6. pEMC-VSV-mScarlet VOC_CTRL # clone 3, looks perfect!

Please let me know if you'd like me to email you the sequence files or alignments.

Cheers,

Jeremy

From: Jeremy Kamil <jkamil@lsuhsc.edu>
Date: Friday, January 22, 2021 at 1:47 PM
To: Mohammed Siddiquey <siddiquey27@gmail.com>, Satoshi Ikegame <satoshi.ikegame@mssm.edu>
Cc: Benhur Lee <benhur.lee@mssm.edu>, Griffin Haas <griffin.haas@icahn.mssm.edu>
Subject: RESULTS B.1.351 and VOC_CTRL Sequencing Summary

Hi Satoshi,

Of the plasmids and bacteria that Siddiquey shipped on Tuesday, here's our results from Genewiz..

These clones look **GOOD or LIKELY GOOD** by Sanger Sequencing.

1. pEMC-VSV-eGFP_501Y.V2 (S African) RBD # clone 1 **#RBD only!** This one will not need to use VOC_CTRL as a control.
2. pEMC-VSV-eGFP FULL 501Y.V2 (S African) # clone 2. This is FULL length clone.. will need VOC_CTRL for best data.
    a. ** I can't read the very end of the gBlock because the Rev primer failed. but it is probably fine!
3. pEMC-VSV-eGFP VOC_CTRL # clone 2
    a. note, SILENT wobble mutation of 3rd position of Ser477 (TCT to TCC).. both codons pretty much equally abundant.. no expected effect!
4. pEMC-VSV-mScarlet VOC_CTRL # clone 3, looks perfect!

These clones are **BAD:**

5. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S

African) # clone 2.

   a. Missense mutation of V610I (numbering per Wuhan Hu-1 REF Spike) …this would be Val606Ile in B.1.351 (501Y.V2) Spike
   b. Silent unintended GGC to GGT wobble mutation at Gly codon 252 (Wuhan REF numbering).. this would be Gly249 of actual 501Y2 translation product.

These clones I can't tell yet..

1. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 1
   a. can't read ATG at 5'-end due to smear.
2. Some others didn't sequence well yet either..

Here is the raw data https://www.dropbox.com/s/2ebdfnpm42rkfgv/JEREMYKAMIL01-22-2021_182552_347.zip?dl=0

And the alignments: https://www.dropbox.com/sh/27uz4816j3xolll/AACc5_7RMFJ8jP_nT4aFAgfsa?dl=0 (some are macvector format but also FASTA are provided).

Cheers,

Jeremy

From: Mohammed Siddiquey <siddiquey27@gmail.com>
Date: Tuesday, January 19, 2021 at 7:06 PM
To: Ikegame, Satoshi <satoshi.ikegame@mssm.edu>
Cc: Lee, Benhur <benhur.lee@mssm.edu>, Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Subject: Re: SARS-CoV-2 RBD in vitro evolution

follows contagious mutation spread, yet generates an able infection inhibitor | bioRxiv

EXTERNAL EMAIL: EVALUATE

Hi Satoshi,

I am sending you the following today. The tracking number is 772672573621.

Please let me know after receiving.

Thank you.

Siddiquey

DNA (20 ul, >100 ng /ul):

1. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 1
2. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 2
3. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 3

4. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 1

5. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 2

6. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 3

7. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 1

8. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 2

9. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 3

10. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 1

11. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 2

12. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 3

13. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 1

14. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 2

15. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 3

16. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 1

17. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 2

18. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 3

19. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 1

20. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 2

21. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 3

Bacteria (50 ul):

22. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 1

23. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 2

24. pEMC-VSV-eGFP D614G_501Y-V2 (S African) RBD # clone 3

25. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 1

26. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 2

27. pEMC-VSV-eGFP D614G_VOC_CTRL # clone 3

28. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 1

29. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 2

30. pEMC-VSV-mScarlet D614G_VOC_CTRL # clone 3

31. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 1

32. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 2

33. pEMC-VSV-E2_Crimson D614G_VOC_CTRL # clone 3

34. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 1

35. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 2

36. pEMC-VSV-eGFP D614G_full 501Y.V2 (S African) # clone 3

37. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 1

38. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 2

39. pEMC-VSV-mScarlet D614G_full 501Y.V2 (S African) # clone 3

40. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 1

41. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 2

42. pEMC-VSV-E2_Crimson D614G_full 501Y.V2 (S African) # clone 3

On Thu, Jan 14, 2021 at 1:49 PM Ikegame, Satoshi <satoshi.ikegame@mssm.edu> wrote:

> I noticed I cannot start transformation until you get sequence result.
>
> Sorry for the confusion.
>
> Satoshi Ikegame
>
> mail to:
>
> satoshi.ikegame@mssm.edu
>
> Department of Microbiology, Icahn School of Medicine at Mount Sinai.
>
> ---
>
> **From:** Ikegame, Satoshi
> **Sent:** Thursday, January 14, 2021 2:46:44 PM
> **To:** Mohammed Siddiquey; Lee, Benhur
> **Cc:** Kamil, Jeremy P.
> **Subject:** Re: SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor | bioRxiv
>
> Hi Shiddiquey.
>
> I just received package and will start transformation.
>
> Thank you.

Next time, could you send us bacteria (20ul LB medium in tube) in addition to plasmid if possible?

Best,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine at Mount Sinai.

From: Mohammed Siddiquey <siddiquey27@gmail.com>
Sent: Wednesday, January 13, 2021 8:46:14 PM
To: Lee, Benhur
Cc: Ikegame, Satoshi; Kamil, Jeremy P.
Subject: Re: SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor | bioRxiv

Hi Satoshi,

I have sent you the following gBlocks and DNA today. Tracking number is 772622125267.

Please let me know after receiving. I am retransforming 417_501 NKY mutant. Colonies from my first transformation are a little weird. Hopefully I can send you some good clones soon.

Thank you.

Siddiquey

gBlocks:

1. Bsu Sph 417_501 NKY (gBlocks, 10 ng/ul), 20 ul
2. B.1.1.7 (gBlock 1, 10 ng/ul) 20 ul
3. B.1.1.7 (gBlock 2, 10 ng/ul) 20 ul

DNA (20 ul, 100 ng /ul):

4. pEMC-VSV-eGFP D614G_B.1.1.7 # clone 1
5. pEMC-VSV-eGFP D614G_B.1.1.7 # clone 2
6. pEMC-VSV-eGFP D614G_B.1.1.7 # clone 3
7. pEMC-VSV-eGFP D614G_B.1.1.7 # clone 4
8. pEMC-VSV-mScarlet D614G_B.1.1.7 # clone 1
9. pEMC-VSV-mScarlet D614G_B.1.1.7 # clone 2
10. pEMC-VSV-mScarlet D614G_B.1.1.7 # clone 3
11. pEMC-VSV-mScarlet D614G_B.1.1.7 # clone 4

12. pEMC-VSV-E2_Crimson D614G_B.1.1.7 # clone 1

13. pEMC-VSV-E2_Crimson D614G_B.1.1.7 # clone 2

On Mon, Jan 11, 2021 at 5:33 PM Lee, Benhur <benhur.lee@mssm.edu> wrote:

> Hi Jeremy,
>
> I got two random cold calls (emails) today asking me if I have the 501.V2 variant: N501Y, K417N, E484K? One was from a colleague with VA connections saying they want to test 55 COVID-19 positive sera.
>
> What the hell are people thinking? That you (we) make & rescue them just for fun so others can test them? (How do they know we're even doing this?)
>
> My answer is going to be "No, but my collaborators *may* have made them and they are testing them on their panel of sera".
>
> Sounds good? We better get this out soon. Meanwhile, I just asked Satoshi to start virus

evolution expt using sera on our rcVSV-CoV2-S. Since we already have the individual N501Y, delta-F140, and E484K *individual* mutants, do you think starting from one of these mutants will be faster?

(**Satoshi, attached is the paper I was talking to you about earlier . . . it took them 5-8 days for each passage and 7 passages (45 days) to get their first mutant and 14 passages (85-95 days) to get a completely resistant virus: delta F140, E484K, 248-KTRNKSTSRRE 11aa insertion. I think we can do each passage at 24-36 hrs and get resistant mutants in 2 weeks, especially since VSV mutates faster than SARS-CoV-2).**

Anyway, seems like everyone is rushing this, no more being number two after all the hard work we all have put into making this system work. BTW, I find it impossible that a 11-aa insertion spontaneously occurs. Don' you think it must be present a very rare frequency or some sort of recombination event occurred?

Should we have a joint call to determine priorities
once your study section is over?

Benhur

Benhur Lee, M.D.
Professor of Microbiology
Ward-Coleman Chair in Microbiology
Icahn School of Medicine at Mount Sinai
One Gustave L Levy Place #1124
New York, NY 10029
Office: (212) 241-2552 | Lab: (212) 241-9960

Lab
Webpage: LeeLabVirus.Host **| Twitter** @VirusWhisperer

On Jan 11, 2021, at 2:56 PM, Ikegame,

Satoshi <satoshi.ikegame@mssm.edu>
wrote:

N501Y, N501Y+E484K have higher affinity.

This cannot be found in my VSV-CoV2spike.

We may need to condider different kind of assay to evaluate S vs ACE2 affinity.

It is good to know that you've already cloned 501V1 and 501V2.

Regards,

Satoshi Ikegame

mail to:

satoshi.ikegame@mssm.edu

Department of Microbiology, Icahn School of Medicine
at Mount Sinai.

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Sent: Monday, January 11, 2021 2:44:40 PM
To: Lee, Benhur; Haas, Griffin (Student); Ikegame, Satoshi; Mohammed Siddiquey; Hung, Chuan-tien; Dragoi, Ana Maria
Subject: SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor | bioRxiv

USE CAUTION: External Message.

https://www.biorxiv.org/content/10.1101/2021.01.06.425392v2

https://twitter.com/_b_meyer/status/1348695525483290625?s=21

“ While the single 501Y mutations causes a ~2.5x higher affinity to Ace2, the double mutation with E484K results in a ~13x higher affinity to Ace2 compared to the WT version.”

<image0.jpeg>

We have this cloned already (as RBD) of S Africa variant B.1.351, in the GFP VSV Spike backbone.

Cheers,

Jeremy

Sent from phone (please forgive typos)

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Dan Snyder <dan@migscenter.com>
Date: 1/27/2021 11:51:45 AM
Subject: Sequencing

Hi Dan,

Looping in our Vice chancellor, Chris Kevil, here. I think everyone agrees that the Hyb Enrichment Seq you guys are doing is excellent / gives such nice results. I'm not sure when / if we may like to try out an ARTIC protocol, but assuming y'all might offer it down the road, please let us know about pricing and availability.

1. It sounds like La Dept of Health might come be able to support some of our sequencing going forward.. maybe only about 200 sequences per month? Dr. Kevil will know more re the details.

2. In CONFIDENCE — preliminary analyses (Pangolin and Spike aa mutation scanning) suggest there may be a few interesting "variants" in the latest 367 sequences you just finished and uploaded to Box. I'm seeing ones with Spike P681H, Q677H, and Q677P, alongside unique constellations of other clade / lineage defining polymorphisms. The first two of those amino acid changes in Spike are already implicated as being "of concern" since these are close to the furin cleavage site aka "polybasic" PRRAR motif. However, I did not see any classic B.1.1.7 / 501.Y2 "UK" variant.. yet. (The UK variant does have P681H.. the COG-UK team call that mutation "Pooh" like as in "Winnie the Pooh," similarly they call D614G, "Doug", and N501Y "Nelly").

My suspicion is that there could just as well be USA homegrown variants could be competing fine against the B.1.1.7. Perhaps explaining why it hasn't begun to take over yet? Shreveport might not be NYC or Los Angeles, but we are along a major travel corridor (I-20 / I-49) so I think we are well poised to pick it up. I'm just glad we are NOT explicitly pre-screening for B.1.1.7 by S amplicon drop out RT-qPCR because that IMO is garbage for Gen-Epi (adds a huge bias and removes ability to detect homegrown variants that might be out competing others).

Anyway, I'll paste in the "PS" section below some Dropbox links to the csv outputs of some crude web tool analyses I ran last night on the data, in case anyone wants a quick peek. At some point, I'm gonna have to start writing like a half dozen manuscripts, and of course you and Vaughn are on all of them.

Cheers,

Jeremy

PS:

https://www.dropbox.com/s/2bbzfgokbb8kwzd/muts_summ_20210127.rtf?dl=0

https://www.dropbox.com/s/5sg7dg8swq806q4/results_Pangolin_COG_UK_20210127.csv?dl=0

https://www.dropbox.com/s/l4hiyii3dwom6zr/myresults.csv?dl=0

Sent from phone (please forgive typos)

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Kevil, Christopher <CKevil@lsuhsc.edu>
Date: 1/27/2021 12:44:29 PM
Subject: Re: Sequencing

---

1. Nope. None of the official S Africa, UK, or Brazil variants... yet! I think we may well already have homegrown ones kicking.. just like 501Y is recurrent in all of those 3 “classic” variants, and D614G clearly does give an advantage.. I think P681H and maybe Q677H are gonna turn out to define advantages l, too.

2. Many —but not all of these —were community samples. I think there were some jail and def some retirement home samples in the mix as well. Which is great! It will be neat to see how the samples change over time from that huge jail outbreak.

3. Similarly.. down the line a bit we may end up with some reinfections in nursing homes because—according to legend- very old folks have poor immune systems. Would be good to keep in touch with those facilities and physicians who care for the patients, and mare sure we maintain good relationships with LDH so they send samples to us to sequence instead of CDC / Helix / Illumina. Promise them co-authorship.. whatever does the trick!!

Cheers,

Jeremy

Sent from phone (please forgive typos)

> On Jan 27, 2021, at 6:36 AM, Kevil, Christopher <CKevil@lsuhsc.edu> wrote:

> So, you didn’t see the UK, South African, or Brazil variant in the last batch, no?
> And these were all positives from the community, correct?
> I agree with you that it’s clear ‘searching’ for a variant is kinda stupid rather than mapping the evolution of it.
> Honestly, this is very cool stuff leading to numerous questions…. You’re correct… it’s a scientific gold mine.
>
> CK
>
> ---
>
> From: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
> Date: Wednesday, January 27, 2021 at 5:51 AM
> To: Dan Snyder <dan@migscenter.com>
> Cc: Vaughn Cooper <vaughn.cooper@pitt.edu>, Chris <CKevil@lsuhsc.edu>
> Subject: Sequencing
>
> Hi Dan,
>
> Looping in our Vice chancellor, Chris Kevil, here. I think everyone agrees that the Hyb Enrichment Seq you guys are doing is excellent / gives such nice results. I’m not sure when / if we may like to try out an ARTIC protocol, but assuming y’all might offer it down the road, please let us know about pricing and availability.
>
> 1. It sounds like La Dept of Health might come be able to support some of our sequencing going forward.. maybe only about 200 sequences per month? Dr. Kevil will know more re the details.

2. In CONFIDENCE — preliminary analyses (Pangolin and Spike aa mutation scanning) suggest there may be a few interesting "variants" in the latest 367 sequences you just finished and uploaded to Box. I'm seeing ones with Spike P681H, Q677H, and Q677P, alongside unique constellations of other clade / lineage defining polymorphisms. The first two of those amino acid changes in Spike are already implicated as being "of concern" since these are close to the furin cleavage site aka "polybasic" PRRAR motif. However, I did not see any classic B1.1.7 / 501.Y2 "UK" variant.. yet. (The UK variant does have P681H.. the COG-UK team call that mutation "Pooh" like as in "Winnie the Pooh," similarly they call D614G, "Doug", and N501Y "Nelly").

My suspicion is that there could just as well be USA homegrown variants could be competing fine against the B.1.1.7. Perhaps explaining why it hasn't begun to take over yet? Shreveport might not be NYC or Los Angeles, but we are along a major travel corridor (I-20 / I-49) so I think we are well poised to pick it up. I'm just glad we are NOT explicitly pre-screening for B.1.1.7 by S amplicon drop out RT-qPCR because that IMO is garbage for Gen-Epi (adds a huge bias and removes ability to detect homegrown variants that might be out competing others).

Anyway, I'll paste in the "PS" section below some Dropbox links to the csv outputs of some crude web tool analyses I ran last night on the data, in case anyone wants a quick peek. At some point, I'm gonna have to start writing like a half dozen manuscripts, and of course you and Vaughn are on all of them.

Cheers,

Jeremy

PS:

https://www.dropbox.com/s/2bbzfgokbb8kwzd/muts_summ_20210127.rtf?dl=0

https://www.dropbox.com/s/5sg7dg8swq806q4/results_Pangolin_COG_UK_20210127.csv?dl=0

https://www.dropbox.com/s/l4hiyii3dwom6zr/myresults.csv?dl=0

Sent from phone (please forgive typos)

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Lee, Benhur <benhur.lee@mssm.edu>
Date: 10/21/2020 6:51:47 PM
Subject: Re: Plates of RNA samples..

Oh man, sorry! That probably was confusing. I was just perhaps over exhuberant / excited about the Spike Tyr144 mutant so was seeing if my friend Nat (who works closely with Baric on CoV drug screens) would be able to hook me up with a BSL-3 system to look at it. Took the opp to share some other interesting mutants that we'd found.

Those were just some RNA plates that have the original sample RNA in them from the delTyr144 CoV2.

You can disregard for now... lol.

Anyway.. sorry to hear about the food poisoning. Glad you're Ok!

Cheers,

Jeremy

Sent on the go. (Please forgive typos)

From: Lee, Benhur <benhur.lee@mssm.edu>
Sent: Wednesday, October 21, 2020 1:48PM
To: Kamil, Jeremy P.
Subject: Re: Plates of RNA samples..

I'm obviously out of the loop . . . had food poisoning on Sunday-Mon, then had tio gte cleared to return to work since diarhhea is one of the symptoms but keith and I could trace it back to a single source of eating expired food. Sigh.

Anyway, I'm not sure what these samples are, or if I am mean to do something.

Benhur

Benhur Lee, M.D.
Professor of Microbiology
Ward-Coleman Chair in Microbiology
Icahn School of Medicine at Mount Sinai
One Gustave L Levy Place #1124
New York, NY 10029
Office: (212) 241-2552 | Lab: (212) 241-9960

Lab Webpage: LeeLabVirus.Host **| Twitter** @VirusWhisperer

> On Oct 16, 2020, at 1:41 PM, Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:
>
> USE CAUTION: External Message.
>
> Hi Dan, (also copying Vaughn and my superb collaborator Benhur Lee so they can have this info, too)
>
> This is great news! Now I'll check if I can find a BSL-3 CoV2 lab that might be willing to transfect them. Attaching a file with the plate and well info + a .gb file that annotates the reference sequence with the mutations.

ONE IDEA.. if we do find a BSL3 collaborator to transfect them, we can even pool together each set of samples that has an identical virus in it, which might help increase success of recovery..

My friend Nat Moorman (Assoc Prof at UNC CH) is in same Dept and collaborates with Ralph Baric and Mark Heise.. They might help us look at some of these in a mouse model… Though he suggested that the UNC folks can just put the mutations into a mouse-adapted infectious clone.

EVTL607 and 614 are identical to each other, so that's like 10-16 uL..
EVTL 410-417 (8 viruses) are identical to each other… so that gives at least 40 uL
EVTL 437-438 are identical too, so that's like 10-16 uL..

Crossing fingers that we can find someone to rescue these.. (assuming that I'm right that this is worth doing .. haha).. seems like a good insurance policy to maximize impact of any epi-papers re these viruses the outbreaks we found them from…

Best,

Jeremy

From: Dan Snyder <dan@migscenter.com>
Date: Friday, October 16, 2020 at 12:01 PM
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Subject: Re: Plates of RNA samples..

EXTERNAL EMAIL: EVALUATE
Hi Jeremy,

We do still have the original RNA plates, which were immediately frozen again after we pulled our aliquot. I would guess we have around 5-8uL or RNA left in each well.

Dan

On Fri, Oct 16, 2020 at 1:36 AM Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:

> Hi Dan,
>
> There are about 12 samples from the most recent set of 4 plates you guys just sequenced from that are potentially worth growing up. Found out that our testing lab tossed out all the remaining original viral transport media samples corresponding to those from the -80 freezers.
>
> Just wondering if you guys still have any of the RNA left … in case I can find a BSL-3 CoV2 collaborator who wants to try to transfect it into VERO cells.
>
> Long shot.. but just curious. (I'm furious that our testing lab threw the original swab + VTM samples away.. Argh!)
>
> Cheers,
>
> Jeremy

> > Sent from phone (please forgive typos)

> --
> Dan Snyder, Director
> Microbial Genome Sequencing Center
> 355 Fifth Avenue, Suite 810
> Pittsburgh, PA 15222
> (412) 475-2318
>
> <Coded Facility name_mutations_20201015 ORF7a Spike etc.xlsx><EPI_ISL_402124 WIV4.gb>

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Steven Meyers <steven@gisaid.org>
Date: 6/26/2020 12:43:08 AM
Subject: Re: Correction to covv_add_location for 3 nCoV-19 EPI_ISL

Wow... so goes beyond Trumpism? So disgusting. Call me sometime I'd love to learn more and not have a paper trail. I'm on Signal too btw! 318.564.7433.

Hope Chris Mooney will tell the tale someday soon!!!

Sent on the go. (Please forgive typos)

From: Steven Meyers <steven@gisaid.org>
Sent: Thursday, June 25, 2020 7:40:37 PM
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Subject: Re: Correction to covv_add_location for 3 nCoV-19 EPI_ISL

It is and never has been about collaboration for these kind of individuals. They wouldn't know how to spell it.

https://ncbiinsights.ncbi.nlm.nih.gov/2020/06/25/new-genbank-submission-options-sars-cov-2/

their deep rooted anger is plainly visible and ironically not a product we can put in the box of this admin or political ideology

---

From: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
Date: Thursday, June 25, 2020 at 15:17
To: GISAID EpiCoV Team <hCoV-19@gisaid.org>
Cc: Steven Meyers <steven@gisaid.org>
Subject: Re: Correction to covv_add_location for 3 nCoV-19 EPI_ISL

Hello EpiCov Team,

(1) Firstly, Thanks! You guys are great.

(2) Secondly, I found a few more EPI_ISL's that had Sex / gender data on the patient missing... here are the sex / gender for a list of Accessions that I think are currently missing those metadata.

EPI_ISL_435555. Sex = Female
EPI_ISL_435557. Sex = Female
EPI_ISL_435558. Sex = Female
EPI_ISL_435560. Sex = Female
EPI_ISL_435561. Sex = Female
EPI_ISL_435562. Sex = Female
EPI_ISL_435563. Sex = Female
EPI_ISL_435564. Sex = Female

(3) Third, thanks for the heads up about the "Additional Location Field". For future uploads I will enter "Parish name" into the main location field. Thanks for updating those records with the Parish name in the Location field instead of the "additional location field"

(4) I am proud to support GISAID on Social Media.. I sincerely believe in the importance of your work. (It's easy to see how much you guys make a difference!!!). I've also been telling a few different journalists (when they call me) about how important GISAID is, and that it's a great story to tell the public about

how open data helps us react to pandemics, and prepare vaccines quickly, etc.. how NIH / NCBI failed to solve the incentive problem raised by Cox and Bogner in that Nature paper back in August of 2006! Sadly, I still don't think USA is trying seriously to solve that problem and I feel like CDC is perhaps trying to "compete" with GISAID instead of cooperate.. which of course would be the better idea… So far spoken to folks from Washington Post, USA Today, and Wired.

(5) I'm of course not at all affiliated with GISAID.. but it kind of pissed me off that the authors of this Nature Medicine paper hardly mention GISAID.. https://www.nature.com/articles/s41591-020-0935-z

Am I misunderstanding something? Look at Figure 1.. it shows big boxes labeled NCBI, ENA, & Pathogenwatch but not GISAID.. the only place you see GISAID is in the legend to fig 1, as a parenthetical "(e.g. GISAID, ViPR)"

At same time, I have a developed a decent rapport with Duncan MacCannell (CDC) – he reached out to me a month or so back, and invited me to join his "CDC Spheres" Slack group.. he is supportive of our team getting some CDC funding to continue sequencing in NW Louisiana.. so I have to be diplomatic when / if I publicly object to this type of stuff if it involves him. On the plus side, I happen to know Duncan seems to be aware that the CDC has "messed up" (egg on their face, big time) but the impression I get (not subtle) is that their / his hands are tied by the highest levels of US Govt (Political Appointees). Similarly, as you probably saw, EcoHealth Alliance / Peter Daszak had their funding for bat coronavirus work in China pulled because of political interference here (disgusting!) and Francis Collins (NIH Director) was powerless to do stop this travesty from happening.. probably because he'd lose his job.

Have a nice evening!

Best,

Jeremy

From: GISAID EpiCoV Team <hCoV-19@gisaid.org>
Date: Thursday, June 25, 2020 at 3:38 PM
To: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
Cc: GISAID EpiCoV Team <hCoV-19@gisaid.org>, Steven Meyers <steven@gisaid.org>
Subject: Re: Correction to covv_add_location for 3 nCoV-19 EPI_ISL

Dear Kamil,

Many thanks for the opportunity to update your records. We noticed on these three cases that you are using the Additional location field. The idea of this field is not to narrow down the geolocation, but to express a characteristic such as a concert hall, a football stadium, or a wholesale market like we have recently seen in Beijing.

We have updated the Location accordingly and hope you are in agreement

Many thanks for your steadfast support of your platform on social media we have recently learned about.

Best wishes

Your GISAID EpiCoV Team

P.S.: Be sure to email us directly with your data needs to this **hcov-19@gisaid** email address. It is dedicated to support Submitters with priority

From: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
Date: Thursday, June 25, 2020 at 13:10
To: GISAID EpiFlu Database Support <service@gisaid.org>, Steven Meyers <steven@gisaid.org>
Subject: Correction to covv_add_location for 3 nCoV-19 EPI_ISL

Hi Steven and GISAID Support Team,

Would you please help me fix an element of metadata for 3 of our uploaded nCoV-19 sequences?

We accidentally populated Incorrect Data for the "covv_add_location" field when we bulk uploaded the nCoV19 sequences for the following EPI_ISL accessions:

EPI_ISL_451274
EPI_ISL_451282
EPI_ISL_451292

The CORRECT value for "covv_add_location" field for all three of these EPI ISL Accession numbers is "Bossier Parish"

At the time of upload, they were incorrectly labeled as "Caddo Parish" due to a data entry error on our end. That has now been rectified.

Apologies for the error and thank you for your help!

Best,

Jeremy

On 6/1/20, 5:02 PM, "GISAID Support Team" <service@gisaid.org> wrote:

> *EXTERNAL EMAIL: EVALUATE*
>
> Dear "Kamil, Jeremy P." <jkamil@lsuhsc.edu>,
>
> Thank you for contacting GISAID support. We will try and respond to all messages within 1 business day. If you are contacting us with a technical question, kindly allow more response time, as we may need to consult with our technical staff.
>
> Kind Regards,
>
> Your GISAID Support Team

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: GISAID EpiCoV Team <hCoV-19@gisaid.org>
Date: 6/25/2020 10:17:45 PM
Subject: Re: Correction to covv_add_location for 3 nCoV-19 EPI_ISL

Hello EpiCov Team,

1. Firstly, Thanks! You guys are great.

2. Secondly, I found a few more EPI_ISL's that had Sex / gender data on the patient missing… here are the sex / gender for a list of Accessions that I think are currently missing those metadata.

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3. Third, thanks for the heads up about the "Additional Location Field". For future uploads I will enter "Parish name" into the main location field. Thanks for updating those records with the Parish name in the Location field instead of the "additional location field"

4. I am proud to support GISAID on Social Media.. I sincerely believe in the importance of your work. (It's easy to see how much you guys make a difference!!!). I've also been telling a few different journalists (when they call me) about how important GISAID is, and that it's a great story to tell the public about how open data helps us react to pandemics, and prepare vaccines quickly, etc.. how NIH / NCBI failed to solve the incentive problem raised by Cox and Bogner in that Nature paper back in August of 2006! Sadly, I still don't think USA is trying seriously to solve that problem and I feel like CDC is perhaps trying to "compete" with GISAID instead of cooperate.. which of course would be the better idea… So far spoken to folks from Washington Post, USA Today, and Wired.

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Have a nice evening!

Best,

Jeremy

From: GISAID EpiCoV Team <hCoV-19@gisaid.org>
Date: Thursday, June 25, 2020 at 3:38 PM
To: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
Cc: GISAID EpiCoV Team <hCoV-19@gisaid.org>, Steven Meyers <steven@gisaid.org>
Subject: Re: Correction to covv_add_location for 3 nCoV-19 EPI_ISL

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Best wishes

Your GISAID EpiCoV Team

P.S.: Be sure to email us directly with your data needs to this **hcov-19@gisaid** email address. It is dedicated to support Submitters with priority

---

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> Date: Thursday, June 25, 2020 at 13:10
> To: GISAID EpiFlu Database Support <service@gisaid.org>, Steven Meyers <steven@gisaid.org>
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> Best,
>
> Jeremy
>
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Thank you for contacting GISAID support. We will try and respond to all messages within 1 business day. If you are contacting us with a technical question, kindly allow more response time, as we may need to consult with our technical staff.

Kind Regards,

Your GISAID Support Team

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Mouton, Drew <dmout6@lsuhsc.edu>
Date: 6/12/2020 8:50:46 AM
Subject: Re: Article: Mouse model of COVID-19

Hi Drew,

Agree with your takes!   Do we need another recombinant mouse with human ACE2?   Could be valuable and Xiaohong Lu can probably make one.   It will take a while and others are doing it. Not bad idea but only worth something if it's gonna be used in experiments... many of which will already be published by the time his mouse is ready. Overall hard to say whether it's worth doing but on its face it's a useful tool to have. Just can't say —genuinely uncertain—whether cost benefit pans out.

That cell paper takes a shortcut. Pretty slick. Adenovirus (Ad) vector transduction of human ACE2 into mice. Making them express human ACE2 without having to create a new mouse line. Basically Ad vectors do a great job carrying genes into whatever cells we need them too.

Ralph Baric's lab meanwhile mutated the SARS-CoV-2 Spike at 2 amino acids so that the virus could use mouse ACE2 for entry. Thereby opening up mice as a model by making that tiny change to the virus (instead of the mouse). That paper came out about a month ago. Slam dunk approach!
https://www.biorxiv.org/content/10.1101/2020.05.06.081497v1

Sent from phone (please forgive typos)

> On Jun 11, 2020, at 4:28 PM, Mouton, Drew <dmout6@lsuhsc.edu> wrote:

> Jeremy: I'm afraid you've now been entered in my rolodex as 'inner circle of scientific advisors.' ☺
>
> (Except, of course, no one actually has a rolodex any more.)
>
> Hoping to get a quick thought: Xiaohong's mouse model for COVID-19 is chasing a need that it appears lots of labs are also chasing (in support of the 145 companies all pursuing CoV2 vaccines). Wondering 1.) if you could give a 'gut check' to whether his is valuable in the face of others, and then 2.) I have to imagine it's only demonstrably valuable if important places are using it, so thought you might have a suggestion for who to send some to for their use.
>
> Here are the head's for Xiaohong's:
>
> ***"A CRISPR/Cas mediated humanized conditional ACE2 BAC transgenic mouse model for understanding disease pathogenesis and testing the efficacy and safety of medical countermeasures for SARS-CoV, SARS-CoV-2, and emerging coronaviruses."***
>
> *"Transgenic animals are in urgent need and high demand for labs and companies to study pathogenesis and to develop countermeasures for SARS-CoV-2 (https://www.nature.com/articles/d41586-020- 00698-x). Multiple transgenic companies are rushing to develop hACE2 transgenics and they are on sale for high prices. However, all the current human ACE2 transgenic mice available and those in the pipeline were all generated using conventional transgenesis using viral or truncated tissue-specific promoters to drive the expression of a human ACE2 cDNA. The mouse model described in our invention is innovative and is technically challenging to generate, and is out of the realm of the possibility of most of the labs and companies. Importantly, our invention aims to model a human disease process in a more sophisticated manner than any existing hACE2 models or models in the pipeline. For example, JAX is producing the K18-hACE2 transgenic using an epithelial cell*

> *promoter. Cyagen is generating a proprietary conventional ROSA26 Knockin (with a hefty price tag of $1300-1500 per mouse, with a minimal 10 mice per order, mice are not ready until at least 4 months later) that express human ACE2 artificially and ubiquitously in every mouse cell. No current animal models and none of these companies ever generated/generating a mouse model using full-length human ACE2 to humanize the whole mouse Ace2 locus. They simply do not know how to do it. This is the major competitive advantage of this invention.*
>
> (More detail is attached if you want it.)
>
> In reading this, I dont have the micro depth to immediately grok the differences to what was just published in Cell yesterday:
>
> https://www.cell.com/cell/pdf/S0092-8674(20)30742-X.pdf?_returnURL=https://linkinghub.elsevier.com/retrieve/pii/S009286742030742X?showall=true
>
> Would you mind giving me a quick impression?
>
> D
>
> > On Jun 10, 2020, at 5:32 PM, Alexander, Jonathan Steven <JAlexa@lsuhsc.edu> wrote:
> >
> > Mouse model of COVID-19, key in accelerating research, developed by Washington U.
> >
> > https://flip.it/Snk7xl
> >
> > Sent from Outlook Mobile
>
> **Drew Mouton**
> **Chief Innovation Officer**
> Office of Research
> LSU Health Shreveport
> 318-675-7016 phone
> 504-975-2003 cell
> dmout6@lsuhsc.edu
>
> *Confidentiality notice: privileged/confidential information may be contained in this message.* ***This correspondence is covered by the electronic communications privacy act, 18 u.s.c. § 2510-2521 and is legally privileged.***
>
> **Drew Mouton**
> **Chief Innovation Officer**
> Office of Research
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>
> <LSUHSC-S-2020-030 - Lu_Excerpt.pdf>

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Courtlin Vinson <courtlinv8@gmail.com>
Date: 6/10/2020 4:09:21 AM
Subject: Virology related jobs in NYC

This (EcoHealth Alliance) is a fantastic organization. You should apply! They do work related to preventing pandemics and viral outbreaks and they have openings right now:

https://www.ecohealthalliance.org/careers

Here’s a tweet about it

https://twitter.com/ecohealthnyc/status/1270004832359809025?s=21

Sent from phone (please forgive typos)

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Lee, Benhur <benhur.lee@mssm.edu>
Date: 6/8/2020 6:01:05 PM
Subject: Re: Quick update re RBD mutants, PBMC, etc

YES

From: "Lee, Benhur" <benhur.lee@mssm.edu>
Date: Monday, June 8, 2020 at 1:00 PM
To: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
Subject: Re: Quick update re RBD mutants, PBMC, etc

you're free for a quck zoom? benhur

#BeSafe #FlattenTheCurve #SocialDistancingWorks

Benhur Lee, M.D.
Professor of Microbiology
Ward-Coleman Chair in Microbiology
Icahn School of Medicine at Mount Sinai
One Gustave L Levy Place #1124
New York, NY 10029
Office: (212) 241-2552 | Lab: (212) 241-9960

Lab Webpage: LeeLabVirus.Host **| Twitter** @VirusWhisperer

> On Jun 8, 2020, at 1:23 PM, Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:
>
> Hey Benhur,
>
> A few quick things/ questions:
>
> 1. For the panel of RBD mutants, would you recommend FOR or AGAINST truncating the cytoplasmic tail of Spike in pCAGGS? Sean Whelan's VSV SARS2 Spike paper seemed to show a great growth advantage after it spontaneously truncated the last 21 aa. (Deletion of 1253-1273). But re-reading some of your other emails it seems like maybe that's not a good plan? I'm confused!
>
> 2. Just so you know... I didn't spill the beans to my friend Vic DeFilippis (OHSU) about all the details of your nanopore partnership, or your elegant FACS methods.... just told him that you had a hook up for free scRNA sequencing of B-cells from SARS2 seropostives. Turns out he's friendly with Bill Messer (a Ralph Baric trainee) who is banking lots of PBMC at OHSU.. in case that's helpful to you he can set up an intro. Anyway, Vic is an ally and friend who I got to know via one of my very best buddies, Chris Benedict (a viral immunologist at La Jolla Institute). So, I think you can trust him in case the connection might be helpful to you.
>
> 3. I got all the wheels turning here toward getting PBMC collection authorized / IRB approved. Even got a "buy-in" from a physician on our IRB board named Connie Arnold here who helps lead outreach / free SARS-CoV-2 testing for underserved communities (FQHCs). Basically, we have three major sources of blood donor: (i) staff at the hospital who have already seroconverted, (ii) the FQHCs / community health centers we serve, and (iii) the convalescent sera donation pipeline via "LifeShare." It will be a bit of IRB paperwork but I'm happy to do that.. it will probably take a couple weeks minimum to get all the approvals in place.. and we will need a DUA with Mt. Sinai, which can also be expedited. If it helps you, I'm happy to lead the charge and start writing IRB protocol! Connie told me she can help me with the consent forms. We can start with BAMBANKER freezing PBMC to ship to. you. But I also have 10XGenomics sales team in my inbox. .so can pursue that

3. independently, too.

Hope you had a good weekend!

Cheers,

Jeremy

From: "Lee, Benhur" <benhur.lee@mssm.edu>
Date: Tuesday, June 2, 2020 at 6:33 PM
To: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
Subject: Re: Spike RBD mutants...

EXTERNAL EMAIL: EVALUATE
GO ahead and order, especially since cloning is your middle name. By the time you have those made,we can decide to either rescue all of them as pseudotypes (since we use Florian's pCAGS gene, it will be perfect), or as replication competent clones in VSV. I'm on the IBC and DURC, this does NOT violate any "gain-of-funtion" studies because, they are ,by definition, naturally occurring mutations.

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BTW, you should get a final draft of our paper to review tomorrow or Thursday. We intend to upload onto BioRxiv on this weekend.

And no, FBI won't come calling, I've ordered sequences that span almost the entire Nipah virus genome and no one cares.

Benhur

#BeSafe #FlattenTheCurve #SocialDistancingWorks

Benhur Lee, M.D.
Professor of Microbiology
Ward-Coleman Chair in Microbiology
Icahn School of Medicine at Mount Sinai
One Gustave L Levy Place #1124
New York, NY 10029
Office: (212) 241-2552 | Lab: (212) 241-9960

Lab Webpage: LeeLabVirus.Host **| Twitter** @VirusWhisperer

> On Jun 2, 2020, at 5:38 PM, Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:
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> Hi Benhur,
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> So, here's a couple of exports from UCSF Chimera.. turns out all the GISAID RBD mutants that show up 2 or more times are between aa 439-494. Pretty small patch of the RBD.. only 56 aa. And... even though there are 23 mutations found so far.. only 12 show up 2 or more times, and only 6 show up 5 or more times..
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The attached Chimera images show this 439-494 patch on the RBD “up,” and another in on an RBD “down” subunit.

Anyway, if you use Florian’s pCAGGS-Spike to pseudotype, there’s an MluI – SphI release-able fragment that could be replaced by IDT gBlocks of 517 bp, even including 20 bp Gibson flanks for assembly.. I already checked and this fragment’s sequence passes their “complexity” filters.. though probably the FBI will visit me once I place an order.

Cheers,

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Mutation	**Occurence**
N439K	154x: UK, Romania
T478I	52x: England
V483A	30x; USA, Englad
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V483F	5x; Spain, UK
A475V	4x; USA, Australia
G446V	3x; Australia, UK
V483I	2x; UK
F490L	2x; Austrialia, USA
F490S	2x; UK
Q493L	2x; USA

<imageSpike RBDdown_439_434.png><imageSpike RBDup_439_434.png>

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Lee, Benhur <benhur.lee@mssm.edu>
Date: 6/8/2020 5:23:36 PM
Subject: Quick update re RBD mutants, PBMC, etc

Hey Benhur,

A few quick things/ questions:

1. For the panel of RBD mutants, would you recommend FOR or AGAINST truncating the cytoplasmic tail of Spike in pCAGGS? Sean Whelan's VSV SARS2 Spike paper seemed to show a great growth advantage after it spontaneously truncated the last 21 aa. (Deletion of 1253-1273). But re-reading some of your other emails it seems like maybe that's not a good plan? I'm confused!

2. Just so you know… I didn't spill the beans to my friend Vic DeFilippis (OHSU) about all the details of your nanopore partnership, or your elegant FACS methods.... just told him that you had a hook up for free scRNA sequencing of B-cells from SARS2 seropostives. Turns out he's friendly with Bill Messer (a Ralph Baric trainee) who is banking lots of PBMC at OHSU.. in case that's helpful to you he can set up an intro. Anyway, Vic is an ally and friend who I got to know via one of my very best buddies, Chris Benedict (a viral immunologist at La Jolla Institute). So, I think you can trust him in case the connection might be helpful to you.

3. I got all the wheels turning here toward getting PBMC collection authorized / IRB approved. Even got a "buy-in" from a physician on our IRB board named Connie Arnold here who helps lead outreach / free SARS-CoV-2 testing for underserved communities (FQHCs). Basically, we have three major sources of blood donor: (i) staff at the hospital who have already seroconverted, (ii) the FQHCs / community health centers we serve, and (iii) the convalescent sera donation pipeline via "LifeShare." It will be a bit of IRB paperwork but I'm happy to do that.. it will probably take a couple weeks minimum to get all the approvals in place.. and we will need a DUA with Mt. Sinai, which can also be expedited. If it helps you, I'm happy to lead the charge and start writing IRB protocol! Connie told me she can help me with the consent forms. We can start with BAMBANKER freezing PBMC to ship to. you. But I also have 10XGenomics sales team in my inbox. .so can pursue that independently, too.

Hope you had a good weekend!

Cheers,

Jeremy

From: "Lee, Benhur" <benhur.lee@mssm.edu>
Date: Tuesday, June 2, 2020 at 6:33 PM
To: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
Subject: Re: Spike RBD mutants...

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From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Cooper, Vaughn S. <vaughn.cooper@pitt.edu>
Heguy, Adriana <Adriana.Heguy@nyulangone.org>
Date: 5/5/2020 12:08:48 PM
Subject: Re: GISAID's preferred Refseq is EPI_ISL_402124 (not Wuhan Hu-1)
Attachments: Screen Shot 2020-05-05 at 7.07.12 AM.png

Hi Adriana and Vaughn,

Sounds like we and/or at least you guys will happen. If there's a role for me to play that'd be genuinely helpful beyond computation / hyb-capture sequencing, please let me know. I'd be totally excited to contribute, and it'd be wonderful to have NIH support more sequencing of the samples we are identifying from our EUA diagnostic facility.

I'd of course like to know more of what your thoughts are, Vaughn, regarding the biology. I'm still at the scratching my head re: what are the impactful / actionable goals aside from just "observing" where variation occurs, how frequently it is happening, and what mechanisms might be at play (evidence of host defense vis a vis APOBECs, vs. random error, roles of selection?).

So you already have a microbial evolution R01 that you would ask for a supplement for? There's also this mechanism, which is not for supplements, but just straight up "Rapid" R01: https://grants.nih.gov/grants/guide/pa-files/PAR-20-178.html

A final thought… and maybe this is naïve, but I the more I look at the mutations that show up across the clades, I wonder why they are almost always C —> T but never (or almost never) T—>C. Could this be reflecting role for APOBEC cytosine deaminases? This is evident even just in a breseq output for any given virus result we compile against EPI_ISL_402124, for example. .screenshot attached. (Not sure why APOBECs wouldn't be operating in the bat hosts though, too.. perhaps a good question for John Schoggins). For some reason—I assume based on evidence, virologists now seem to assume APOBECs mostly act on DNA viruses or retroviruses which go through DNA intermediate, they were originally discovered as mRNA editing enzymes. I saw one Scientific Reports paper from 2018 with Volker Thiel on it that showed activity of APOBECs on Coronaviruses but they didn't see "hypermutation." https://www.nature.com/articles/s41598-018-24448-2

Cheers,

Jeremy

PS: Vaughn, I didn't realize you had such a strong Virology pedigree or that you'd worked on VSV in the past. Very cool.

--
Jeremy P. Kamil, Ph.D.
Associate Professor
Microbiology and Immunology
LSU Health Sciences Center, Shreveport

318.564.7433 (mobile)
318.675.5771 (office)

From: "Cooper, Vaughn S." <vaughn.cooper@pitt.edu>
Date: Monday, May 4, 2020 at 8:18 PM
To: "Heguy, Adriana" <Adriana.Heguy@nyulangone.org>
Cc: Jeremy Kamil <jkamil@lsuhsc.edu>
Subject: Re: GISAID's preferred Refseq is EPI_ISL_402124 (not Wuhan Hu-1)

Hi Adriana and Jeremy,

Thanks for the intro and great response.

(Switching to my Pitt account and my academic research hat. We're super proud of MiGS but it's primarily a sequencing biz.)

I've been studying pathogen evolution and thinking about genetic diversity within hosts since my undergrad research >25 years ago (eventually published here) — when I was a budding virologist. I then worked on VSV with John Holland and then left and became a bacteriologist w/ Rich Lenski, and afterwards it's been mostly bacterial evolution of AMR and during chronic infections, then a recent foray into cancer evolution like you Adriana, — and then SARS-CoV-2 happened.

I would be THRILLED to work with you to look for systematic, parallel patterns of within-patient diversity in your samples as well as ours (Pitt, LSU). There are at least two approaches:

1) I send you scripts / software and you have someone mine your FASTQ's for this information or
2) You send us FASTQ's and we do the same.

If either work, I would like to propose a rapid response R01 supplement to do this.

What do you think? Can we make this happen? We will need 1000's of genomes to do this.

Best
Vaughn

Vaughn Cooper, Ph.D. Professor
Microbiology and Molecular Genetics | Computational and Systems Biology | Biological Sciences
Director, Center for Evolutionary Biology and Medicine
University of Pittsburgh, School of Medicine
425 Bridgeside Point II, 450 Technology Drive Pittsburgh, PA 15219
m: 603.988.7590 o: 412-624-1265
lab | outreach | microbial genomics facility

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> From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
> Sent: Monday, May 4, 2020 5:43 PM
> To: Heguy, Adriana

Cc: Vaughn Cooper
Subject: Re: GISAID's preferred Refseq is EPI_ISL_402124 (not Wuhan Hu-1)

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IN ANY CASE: If you and Vaughn hit it off and end up being able to work together to answer some questions – or just to extract more public health / basic science value out of the huge amounts of NGS data you have sitting around, that'd be great. No obligation to involve me!

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Viruses are super fascinating!

Cheers,

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Date: Monday, May 4, 2020 at 4:01 PM
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Following up — yes, I'll take your offer to make the connection with Heguy re: evolution within infections. I'm interested!

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>
> Best,
>
> Jeremy

Sent from phone (please forgive typos)

> On May 1, 2020, at 9:45 PM, Vaughn Cooper <vaughn@migscenter.com> wrote:
>
> *EXTERNAL EMAIL: EVALUATE*
> Wow, Jeremy, you are hustling! Thanks for all of this backstory. Very valuable.
>
> Do you need us to reassemble against the bona fide reference?
> We noted the BaseSpace tool but were unsure if it passed muster. If they prefer, happy to use it.
>
> Finally, thanks for the continued support on the twitters. Dan and I owe you some swag!
>
> Cheers
> Vaughn
>
>> On May 1, 2020, at 9:20 PM, Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:
>>
>> Learned some useful stuff from a GISAID representative named Steven Meyers (a German based in Santa Monica.. Lucky guy), who phoned me shortly after I submitted our data.. (mostly to correct a field I inadvertently left empty). Learned lots of things from him.. He gave me the whole backstory of GIAID – Nancy Cox (CDC) and Peter Bogner (his boss) built GISAID.. for Flu.. and then along comes this..and China asked for their help in Dec or Jan or whatever..
>>
>> Important point to share now.. GISAID actually aligns / recommends assemblies be done against EPI_ISL_402124. This is the authentic, traceable to a person and sample, first index case of the pneumonia in China. (Apparently NCBI is Crap for SARS-CoV-2.. Wuhan Hu-1 not traceable to a lab or person.. maybe I'm explaining it wrong but they don't like that file. Haha!
>>
>> Other tidbits.:
>> VAUGHN..
>> (1) They were SUPER impressed by your coverage. Steven asked if some were typos (extra digit?).. they were not.
>> (2) if you use BaseSpace there's now a tool to directly assemble a SARS-CoV-2 genome out of the raw data (fastq.gz files)..
>> (3) he also told me a lot about the evolution from Horseshoe bats, which came from bats who roost in caves in jungle near Vietnam (if I remember what he said, correctly).

(4) Learned many wonderful things about GISAID..

they lock people out who don't acknowledge / cite the sequences they publish with! They are all about people getting credit for what they do.

they are going to make "EpiSet" files to allow others to download the same exact set of sequences that a person / publication uses in an analysis..

they are about to update their software to allow us to upload the entire raw data (reads) that were used to build a FASTA file !

Best,

Jeremy

Jeremy P. Kamil, Ph.D.
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<EPI_ISL_402124.pdf><EPI_ISL_402124.fasta>

Vaughn Cooper, Ph.D.
Chief Scientific Officer
vaughn@migscenter.com

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Nat Moorman <nathaniel_moorman@med.unc.edu>
Date: 5/4/2020 10:35:00 PM
Subject: FW: GISAID's preferred Refseq is EPI_ISL_402124 (not Wuhan Hu-1)

Hi Nat.. please forward the words below to Ralph. This is from his old friend –Adriana Heguy—from when he was a postdoc at USC. She is a cancer biologist who in fact ended up getting sucked into COVID19 big time and has done about 500 or more SARS2 genomes already on GISAID. She's not quite Ralph level but is in this NYTimes article by Carl Zimmer showing that the viruses in NYC came from Europe.

https://www.nytimes.com/2020/04/08/science/new-york-coronavirus-cases-europe-genomes.html

I got to know her a bit via Twitter.. she is awesome. You can see I name dropped you below.

Cheers,

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Finally, thanks for the continued support on the twitters. Dan and I owe you some swag!

Cheers
Vaughn

> On May 1, 2020, at 9:20 PM, Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:
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Best,

Jeremy

Jeremy P. Kamil, Ph.D.
Associate Professor
Dept. of Microbiology and Immunology
LSU Health Sciences Center, Shreveport
Shreveport, Louisiana, USA
Ph: +1.318.759.7544
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<EPI_ISL_402124.pdf><EPI_ISL_402124.fasta>

Vaughn Cooper, Ph.D.
Chief Scientific Officer
vaughn@migscenter.com

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Adriana Heguy <Adriana.Heguy@nyulangone.org>
Date: 5/4/2020 9:43:54 PM
Subject: Re: GISAID's preferred Refseq is EPI_ISL_402124 (not Wuhan Hu-1)

Hi Adriana,

Introducing you to Vaughn Cooper, who has a lot of experience studying evolution of microbial genomes. Vaughn is curious whether there might be some meaning underneath certain of the "mixed variants" that show up in Illumina data from SARS-CoV-2 clinical samples.. Vaughn completely gets that most variants are noise / not of great meaning. I'm sure that we are all also well aware that RNA viruses are sloppy copiers / generate quasispecies – but also that CoV's encode an RNA replicase proofreading function/ nsp14, which allows for their "larger than the average RNA virus"-sized genome. But if you watch his youtube video (10 min), he uses the classic WWII fighter plane Survivorship Bias analogy to argue that regions of the virus that are underrepresented for SNPs might provide clues to new drug / therapeutic targets. https://www.youtube.com/watch?v=7gCY9tP981I

IN ANY CASE: If you and Vaughn hit it off and end up being able to work together to answer some questions – or just to extract more public health / basic science value out of the huge amounts of NGS data you have sitting around, that'd be great. No obligation to involve me!

For my part, I do find it intriguing that certain viral genes might accumulate variants that approach50% of the reads. I'd like to known if any of these show up often and are well represented already in extant viruses on GISAID. Most these SNPs are no doubt noise / neutral / no effect on fitness.. but if by looking at lots of data sets you can begin to see ones that keep popping up over and over again, and are not explained away as artifacts of CoV RNA replication mechanisms, it would seem important to understand the biology.

(i) Host encoded factors, like APOBEC, mutate HIV RNA by cytidine deamination. Are host innate factors that do something similar against SARS2? Can you infer anything in high % read SNPs about host factors that might target the viral genome?
(ii) Certain SARS2 mutations might facilitate intrahost spread.. but are deleterious for horizontal transmission, so are there certain SNPs keep popping up in NP swabs as a decent sized minority of reads, but which are not maintained long term –i.e. underrepresented in consensus assemblies on GISAID

Although I'm just an "a la carte" human cytomegalovirus (herpes) virologist, who tends to chase sparkly things like a crow would.. I do have excellent friends in the Coronavirus community (e.g. Tony Fehr, at KU and my friend / collaborator Nat Moorman is in Ralph Baric's dept and collaborates extensively with Ralph).. so if you guys end up generating ideas from your combined data that need to be tested on the bench, I can certainly help make further introductions.

Viruses are super fascinating!

Cheers,

Jeremy

From: Vaughn Cooper <vaughn@migscenter.com>
Date: Monday, May 4, 2020 at 4:01 PM
To: Jeremy Kamil <jkamil@lsuhsc.edu>
Subject: Re: GISAID's preferred Refseq is EPI_ISL_402124 (not Wuhan Hu-1)

Following up — yes, I'll take your offer to make the connection with Heguy re: evolution within infections. I'm interested!

VC

On May 3, 2020, at 3:32 PM, Kamil, Jeremy P. <jkamil@lsuhsc.edu> wrote:

So, Adriana Heguy at NYU Languone— she has about 500 genomes done already— told me that they too have evidence of “mixed variants” during infections... Since you and she both run big sequencing centers / projects, maybe I can introduce you to each other? (In the event y’all aren’t already in touch).

(I got to know her because she shared her hyb capture protocols with me way back in mid March, when I was first looking into how to get get this done.)

Anyway, Vaughn, I’d love to see someone with your firepower / experience lead a study looking at this stuff. Also— guessing you’ve seen GISAID report from today that Steven got us both added to.. (from Cheryl Bennett, with Peter Bogner on the cc line!). If I understand the GISAID email correctly, there might actually be a minor number of mutants, say in Spike, that may affect transmission (F456L). (Caution, I know, is warranted). So I interesting to think of you and Adriana pooling primary / raw data from a ton of samples, to ask whatever questions you might be most interested in.

Apologies if you’re already 15 steps ahead of my thoughts here. :-).

Finally, I am also friendly with Benhur Lee, who could directly assay using VSV pseudovirions if any variants affect entry. (He may already be doing this!).

I’m also in contact within some folks in George Church’s lab.. they have a team of people developing LAMP-Seq, basically using RT-LAMP with incorporation of NGS for bar codes to enable massively parallel diagnostics using Illumina platforms. They will be getting sequence data when they make a call on a patient result (+ vs - for SARS) in a pooled run. So if there are regions of special interest for clades, or even possible Spike polymorphisms, they might benefit from hearing from you or lol.. simply looking at GISAID...

surprisingly few people are integrating the molecular epidemiology and basic virology + diagnostics etc!

Please let me know if you’d like me to put you in touch with anyone....

Best,

Jeremy

Sent from phone (please forgive typos)

> On May 1, 2020, at 9:45 PM, Vaughn Cooper <vaughn@migscenter.com> wrote:
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> *EXTERNAL EMAIL: EVALUATE*
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Jeremy P. Kamil, Ph.D.
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<EPI_ISL_402124.pdf><EPI_ISL_402124.fasta>

Vaughn Cooper, Ph.D.
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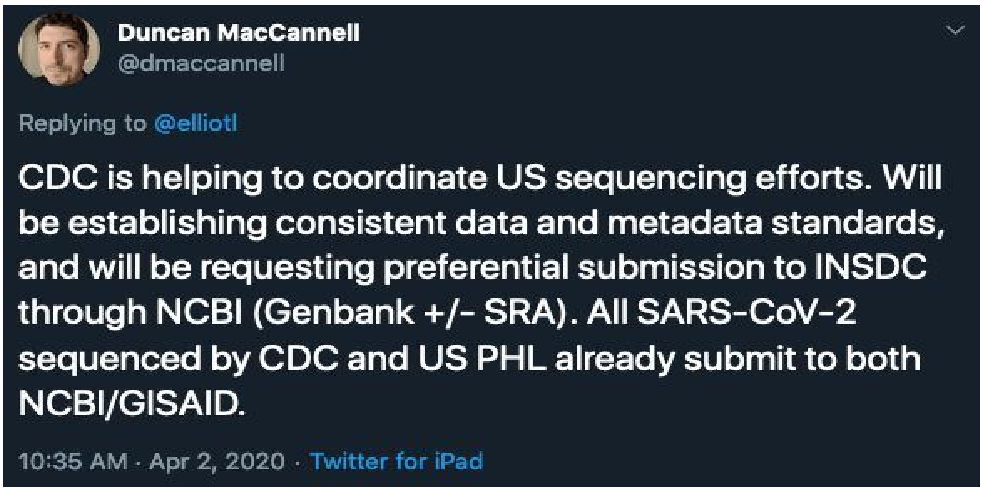
From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Steven Meyers <steven@gisaid.org>
Date: 5/4/2020 4:03:18 AM
Subject: Re: hCoV-19/USA/LA-EVTL011/2020 EPI_ISL_435044

What a tool.   They should work with GISAID instead of trying to compete.

Thanks for showing me this. I'm gonna look him / this up on Twitter.

Sent on the go. (Please forgive typos)

From: Steven Meyers <steven@gisaid.org>
Sent: Sunday, May 3, 2020 11:00:57 PM
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Subject: Re: hCoV-19/USA/LA-EVTL011/2020 EPI_ISL_435044



Duncan MacCannell
@dmaccannell

Replying to @elliotl

CDC is helping to coordinate US sequencing efforts. Will be establishing consistent data and metadata standards, and will be requesting preferential submission to INSDC through NCBI (Genbank +/- SRA). All SARS-CoV-2 sequenced by CDC and US PHL already submit to both NCBI/GISAID.

10:35 AM · Apr 2, 2020 · Twitter for iPad

From: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
Date: Sunday, May 3, 2020 at 23:49
To: Steven Meyers <steven@gisaid.org>
Subject: Re: hCoV-19/USA/LA-EVTL011/2020 EPI_ISL_435044

That's disgusting. Never heard of Duncan. But already a lost cause, the ship has sailed.

We have 30 more sequences ready for upload. Headed your way tomorrow as soon as I have info on some other hard working folks in the diagnostic lab who will be added as coauthors this time.

Btw, I'm (cell) +1-318.564.7433 on Signal or Jeremy.kamil@gmail.com, too.

Thanks for the heads up re Duncan.

Hats off to you guys! And thank you again for adding me to that daily briefing email. Very grateful.

Best,

Jeremy

Sent on the go. (Please forgive typos)

From: Steven Meyers <steven@gisaid.org>
Sent: Sunday, May 3, 2020 10:41:47 PM
To: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Subject: Re: hCoV-19/USA/LA-EVTL011/2020 EPI_ISL_435044

Hi Jeremy

Looks like this Duncan guy from CDC is really trying to go after GISAID after all, trying to persuade Submitters that voluntarily chose to share their data under the GISAID mechanism, to upload to Genbank. Of course, suggesting they can also upload to GISAID, is dodging the issue that he does not want GISAID to exist anymore.

Scary times to be messing with the sharing in the middle of a pandemic, just because one can!

Steven

From: "Kamil, Jeremy P." <jkamil@lsuhsc.edu>
Date: Saturday, May 2, 2020 at 12:49
To: Steven Meyers <steven@gisaid.org>
Subject: Re: hCoV-19/USA/LA-EVTL011/2020 EPI_ISL_435044

Likewise! Was a trip talking to you, Steven.

Hope to buy you a drink when this is over.. Thanks, also, for adding Vaughn and I to that daily update from Cheryl Bennett. Very fascinating stuff.

I enjoyed and appreciate the dendrogram re: relatedness to the Bat virus (EPI_412977). I am so low tech I still align stuff manually with ClustalW in MacVector. But it will be easy to align EPI_ISL_412977 to 402124, and I'm going to do that .. right now

Btw, another friend / collaborator of mine is Nat Moorman (from cytomegalovirus field) .. He is in Ralph Baric's Dept. . I do remember early on seeing some of the stories about relatedness to bat virus but some conjecture about intermediate hosts (pangolins, civets). Lots of good science writing about this. The explosion of information is astounding to say the least. Bizarre pathogenesis. Incredible the hubris...people were saying up til very recently that virology was a "dying field"

Have a nice afternoon and a pleasant weekend.

Cheers,

Jeremy

--

From: Steven Meyers <steven@gisaid.org>
Date: Friday, May 1, 2020 at 10:04 PM
To: Jeremy Kamil <jkamil@lsuhsc.edu>
Subject: Re: hCoV-19/USA/LA-EVTL011/2020 EPI_ISL_435044

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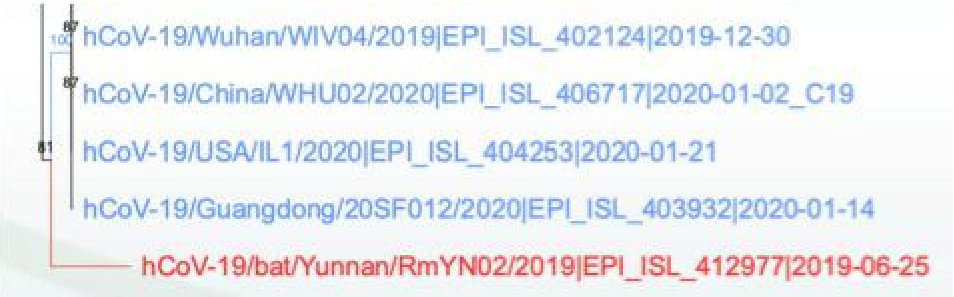
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hCoV-19/USA/IL1/2020|EPI_ISL_404253|2020-01-21
hCoV-19/Guangdong/20SF012/2020|EPI_ISL_403932|2020-01-14
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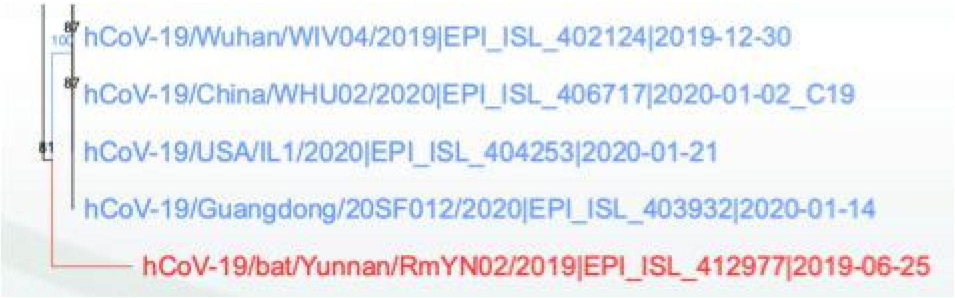
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From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Steven Meyers <steven@gisaid.org>
Date: 5/2/2020 7:49:38 PM
Subject: Re: hCoV-19/USA/LA-EVTL011/2020 EPI_ISL_435044

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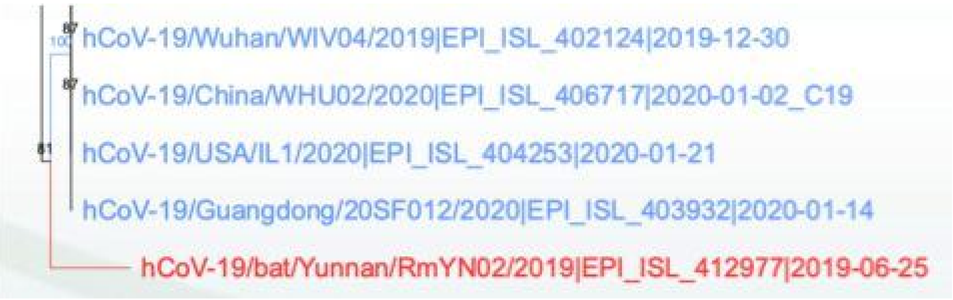
100
hCoV-19/Wuhan/WIV04/2019|EPI_ISL_402124|2019-12-30
hCoV-19/China/WHU02/2020|EPI_ISL_406717|2020-01-02_C19
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From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Kevil, Christopher <CKevil@lsuhsc.edu>
Date: 4/20/2020 6:02:01 PM
Subject: Re: Covid19 and endothelial cells

Crazy idea, perhaps—but if you listen to Ralph Baric (Coronavirus Kingmaker / big wig at UNC) he says that SARS2 will eventually become a circulating common cold virus, much like the others, which he thinks also began as zoonotic leaps that led to pandemics, epidemics.. So… is it possible that some amount of cardiovascular disease / deaths every year, especially in elderly, comes from some of these "Common Cold" Coronaviruses doing unusual things to endothelial cells, heart, etc?

From: "Kevil, Christopher" <CKevil@lsuhsc.edu>
Date: Saturday, April 18, 2020 at 3:30 PM
To: Wayne Orr <aorr@lsuhsc.edu>, "Bhuiyan, Md Shenuarin" <mbhuiy@lsuhsc.edu>, "Kolluru, Gopi K." <gkollu@lsuhsc.edu>, Matthew Woolard <mwoola@lsuhsc.edu>, "Alexander, Jonathan Steven" <JAlexa@lsuhsc.edu>, "Bailey, Steven R." <sbail8@lsuhsc.edu>, "Dominic, Paari" <pdomi2@lsuhsc.edu>, Martin Sapp <MSapp1@lsuhsc.edu>, Andrew Yurochko <AYuroc@lsuhsc.edu>, Jeremy Kamil <jkamil@lsuhsc.edu>, John Vanchiere <jvanch@lsuhsc.edu>, "King, Judy A." <jkin12@lsuhsc.edu>, "Traylor, James" <JTrayl1@lsuhsc.edu>, Paul Weinberger <pweinb@lsuhsc.edu>, "Ghali, Ghali" <GGhali@lsuhsc.edu>, "Shackelford, Rodney E." <rshack@lsuhsc.edu>, "Cotelingam, James" <JCotel@lsuhsc.edu>, "Scott, Louie K." <lscott2@lsuhsc.edu>, "Conrad, Steven" <SConrad@lsuhsc.edu>, "Walter, Robert E." <rwalt1@lsuhsc.edu>, "Holladay, Robert C." <rholl3@lsuhsc.edu>, "Grier, Laurie" <LGrier@lsuhsc.edu>
Subject: Covid19 and endothelial cells

May help provide a clue why HTN is the leading risk factor for severe covid-19 disease.

All the more urgency for getting into the BSL3 for pivotal in vitro cardiovascular studies.

Thx Jeremy Kamil for the catch and notice!

CK

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Yurochko, Andrew D <AYuroc@lsuhsc.edu>
Date: 4/17/2020 7:12:29 PM
Subject: Re: LSUHS Business Intelligence Team COVID-19 Situation Update

---

Yes. I have a personal one and a slightly more "professional" one for my science identity.. which is the one that I try to keep mostly focused on science / not politics.

Harmit Malik, Akiko Iwasaki, Florian Krammer, Benhur Lee and others give good examples how to make the most of Twitter. Their feeds are more human than professional..

@ Baric_Lab is Ralph Baric's group.. but it's a pretty sleepy Twitter feed.

Blossom Damania has a very good Twitter. @ DamaniaLab

Lab ones are great.. but it's hard to keep them fresh, unless your postdocs or grad students are gonna do that for you.

Best,

Jeremy

On 4/17/20, 1:44 PM, "Yurochko, Andrew" <AYuroc@lsuhsc.edu> wrote:

That does rock. Lets put it on our web site and twitter feed and work out some plan where we update every few days or whatever is appropriate.

On the twitter side; do you have both a professional twitter account and a personal one? I was going to go with Yurochko Lab to start and use that to see the good stuff and then when I =get a hang of it, maybe a personal one.

Andrew D. Yurochko, Ph.D.,
Professor and Carroll Feist Endowed Chair in Viral Oncology,
Vice Chairman, Dept of Microbiology and Immunology,
Dir. Research, Feist-Weiller Cancer Center,
Dir. Research, Center of Excellence for Arthritis and Rheumatology,
Louisiana State University Health Sciences Center,
1501 Kings Highway,
Shreveport, LA 71130-3932.
Phone: 318-675-8332
Fax: 318-675-5764
Email: ayuroc@lsuhsc.edu
Website: https://nam01.safelinks.protection.outlook.com/?url=http%3A%2F%2Fwww.lsuhscshreveport.edu%2Fmicrobiology&amp;data=02%7C01%7Cjkamil%40lsuhsc.edu%7C1e2ef89dc4b54391c12608d7e2ff4b2f%7C3406368982d44e89a3281ab79cc58d9d%7C0%7C0%7C637227458422889953&amp;sdata=Y7xS7ZegfcOTHRheMl3jZ1nRrXmBFJylcWrDd%2BOuGLw%3D&amp;reserved=0 <https://nam01.safelinks.protection.outlook.com/?url=http%3A%2F%2Fwww.lsuhscshreveport.edu%2Fmicrobiology&amp;data=02%7C01%7Cjkamil%40lsuhsc.edu%7C1e2ef89dc4b54391c12608d7e2ff4b2f%7C3406368982d44e89a3281ab79cc58d9d%7C0%7C0%7C637227458422889953&amp;sdata=Y7xS7ZegfcOTHRheMl3jZ1nRrXmBFJylcWrDd%2BOuGLw%3D&amp;reserved=0>

On 4/17/20, 1:21 PM, "SHVMS Business Intel Team" <shvmsbit@lsuhsc.edu> wrote:

On behalf of the LSUHS Business Intelligence Team, here is an LSUHS COVID-19 situation update.

Please review this report. Your evaluation and feedback will help our team provide the most useful information for you and your colleagues.

Feel free to contact any member of the BI Team if you have any questions or trouble opening the attached report. Your feedback & help in this process if greatly appreciated.

Have a wonderful day!

LSU Business Intelligence Team
shvmsbit@lsuhsc.edu
Admin Building, 3rd Floor, Rm: 321
1501 Kings Hwy Shreveport, LA 71103

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Woolard, Matthew D. <mwoola@lsuhsc.edu>
Date: 4/13/2020 2:58:22 PM
Subject: Re: Questions about BSL3

Hey Matt!

I’m guessing Nat isn’t the person to ask — I am pretty sure he doesn’t do any BSL3 in his own lab... but he could connect you to Timothy Sheahan, would probably know better.

https://sph.unc.edu/adv_profile/timothy-sheahan-phd/

Only issue is that all those Ralph Baric affiliated people are probably super busy right now though! They prob have more email from reporters right now than they do scientific colleagues. Lol.

We also recently made some new friends at UTMB Galveston, like Bob Cross, who are also starting to do a bit of work on SARS2. Want me to see if I can put you in touch with other folks who might be able to answer your questions?

Best,

Jeremy

Sent from phone (please forgive typos)

> On Apr 13, 2020, at 8:39 AM, Woolard, Matthew D. <mwoola@lsuhsc.edu> wrote:

> Hello Nat,
>
> I am working at LSUHSC Shreveport on some COVID-19 and Cardiovascular related issues. Well specifically my school is. They are wanting to start up some work in our BSL3 facility. I have been tasked with over seeing this operation. I have experience working in the BSL3 using Francisella tularensis, just never worked with a virus. My questions relates to determining neutralization in order to bring samples out of the BSL3. They are wanting to bring out protein samples at this time. SO for bacteria this is easy, I just plated samples onto agar plates a couple of times and showed no bacterial growth. How is this done for virus, since the lysing medium will obviously kill cells that would be used for a plaque assay?
>
> Any advice or guidance would be helpful,
>
> I am attaching Jeremy Kamil on this as he is a mutual colleague
>
> Thank you
>
> Matthew D. Woolard, Ph.D.
>
> O'Callaghan Family Endowed Professor in Microbiology
>
> Associate Professor
>
> Department of Microbiology and Immunology

Louisiana State University Health Sciences Center at Shreveport

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mwoola@lsuhsc.edu

From: Kamil, Jeremy P. <jkamil@lsuhsc.edu>
To: Hill, William <hillwi@musc.edu>
Paul Weinberger <paul.weinberger@ochsnerlsuhs.org>
Date: 4/12/2020 9:06:56 PM
Subject: Re: BSL3

Timothy and I know each other — from Twitter and from mutual friends, like Nat Moorman, a collaborator of mine there who together with Baric and Mark Heise just got a $125M initiative launched called “READDI” to do drug screening against future SARS-like viruses, etc.

Timothy is literally on TV these days!!

Great idea to get help from people in that Dept.. they are wonderful.

Best,

Jeremy

Sent on the go. (Please forgive typos)

From: Hill, William <hillwi@musc.edu>
Sent: Sunday, April 12, 2020 4:01:32PM
To: Paul Weinberger <paul.weinberger@ochsnerlsuhs.org>; Kamil, Jeremy P. <jkamil@lsuhsc.edu>
Subject: BSL3

EXTERNAL EMAIL: EVALUATE
Guys Eric Meissner just emailed me:

“I have a friend at UNC that works on coronaviruses and has BSL3 capacity, but they are booked 10 times over at the moment. Maybe if your LSU BSL2 work goes well than I’d be happy to make the connect with UNC and see where things are at? Timothy Sheahan is my friend there, he works with Ralph Baric’s group“

So if we start to see anything positive we may have an in at the next level.

Dave

William D. Hill, Ph.D.
Sent from my iPhone